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Changes in taxonomic, functional and phylogenetic diversity of the fish assemblage in a temperate estuary

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“No one would protect what they don’t care about, and no one would care about what they have never experienced”

David Attenborough

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Abstract

Estuaries are transitional areas, with high importance for numerous fish species which use estuaries as nursery, migration, feeding and spawning grounds. Due to their location, high productivity and economic importance of their species, estuaries have been highly explored by humans, which together with climate change, makes estuaries highly threatened ecosystems worldwide.

Biodiversity is a natural valuable resource at both economic and cultural levels and a useful ecological indicator. It is a wide concept that includes multiple dimensions (taxonomic, functional and phylogenetic).

The main goal of this study was to assess inter-annual, seasonal and spatial changes in biodiversity of the fish assemblage of the Mondego estuary and links with environmental variables. To do so, we used a time series with periodic sampling between 2003 and 2013 reporting fish species richness and abundance, and environmental variables (temperature, salinity, oxygen, runoff, precipitation and the *North Atlantic Oscilation* (NAO) index. Each species was classified taxonomically, and also based on five functional traits (salinity preference, mobility, diet, feeding mode and maximum body length) and on two mitochondrial genes (16s and COI). To investigate changes regarding the fish assemblages, we used several diversity indices (taxonomic – species richness, Shannon-Wiener, Simpson and Pielou's evenness; functional – functionally singular species, functional richness, functional evenness, functional divergence, functional dispersion and functional Rao's Quadratic Entropy; and phylogenetic – phylogenetic Rao's Quadratic Entropy and mean pairwise distance). Moreover, to allow direct comparisons between diversity dimensions we used Rao's Quadratic Entropy.

Regarding environmental variables, our results are in agreement with previous studies, where salinity (direct relationship), temperature (direct relationship) and river runoff (inverse relationship) were the factors with higher influence on the fish assemblages. Moreover, our results highlight the stability of the fish assemblages in the Mondego estuary, with no linear changes in biodiversity facets throughout the study period (with the exception of slight loss of functional richness, which is probably related with the disappearance of freshwater species due to an increase in salinity), despite seasonal variation in taxonomic evenness and inter-annual variation in many indices. Seasonal variations in taxonomic evenness (which occurred in summer) may be explained by the reproductive biology of fishes and the associated recruitment patterns and spawning migrations, since juveniles of most of the fishes that use this estuary as a nursery area attain high densities inside the estuary during spring/summer. There was a spatial gradient (probably caused by salinity) from upstream to downstream of the estuary, with species and functional richness higher downstream and lower upstream whilst evenness was lower downstream and higher in middle areas. Phylogenetic diversity was lower in middle areas than upstream and downstream, which is probably related to higher diversity that exists in the freshwater and marine adventitious areas than in estuarine areas.

Key-words: fish assemblages, biodiversity, Mondego, functional traits, environmental effects

Resumo

Os estuários são áreas de transição entre os rios e os mares, extremamente importantes para as comunidades de peixes, que utilizam os estuários para se alimentarem, como áreas de viveiro e como parte das suas rotas de migração. Devido à alta produtividade que é característica dos estuários e à elevada importância económica das suas espécies, as áreas estuarinas têm sido altamente exploradas pelo Homem. Esta sobre-exploração juntamente com as alterações climáticas, fazem dos estuários um dos ecossistemas mais ameaçados a nível global.

A biodiversidade é um recurso natural valioso, quer a nível económico como cultural, e é também um excelente indicador ecológico. É também um conceito muito vasto, que engloba três vertentes (taxonómica, funcional e filogenética).

O principal objetivo deste trabalho foi investigar a variabilidade espacial, sazonal e temporal das comunidades de peixes do estuário do Mondego, bem como a influência das variáveis ambientais na comunidade de peixes. Para cumprir este objetivo, utilizámos uma série temporal, com amostragens periódicas efetuadas no estuário, entre 2003 e 2013, com informações sobre a riqueza específica, as respetivas abundâncias de cada espécie em cada sessão de amostragem e os valores das variáveis ambientais (temperatura, salinidade, oxigénio, curso de água, precipitação e o índice *North Atlantic Oscilation* (NAO). Cada espécie foi classificada a nível taxonómico e de acordo com cinco características funcionais (preferência de salinidade, mobilidade, dieta, modo de alimentação e tamanho máximo) e dois genes mitocondriais (16s e COI). Para investigar as alterações nas comunidades de peixes do estuário, foram utilizados diversos índices de cada vertente da diversidade (taxonómica – riqueza específica, Shannon-Wiener, Simpson e Pielou; funcional – espécies funcionalmente únicas, riqueza funcional, equitabilidade funcional, divergência funcional, dispersão funcional e índice de Rao; e filogenética – índice de Rao e distância mínima entre pares de espécies). Para além disso, foi feita uma comparação direta entre as três vertentes da diversidade, utilizando o índice de Rao, uma vez que este índice permite fazer uma comparação entre vertentes distintas.

Em relação às variáveis ambientais, os nossos resultados estão de acordo com outros estudos já realizados, onde a salinidade (relação direta), a temperatura (relação direta) e o caudal de água doce (relação inversa), foram os fatores que mais influenciam as comunidades de peixes. Para além disto, os nossos resultados, destacam a estabilidade das comunidades de peixes do estuário do Mondego, uma vez que não existiram diminuições de diversidade em nenhuma das três vertentes (com exceção da riqueza funcional, que está provavelmente relacionado com o desaparecimento de várias espécies de água doce devido a um aumento da salinidade), apesar de variações sazonais ao nível das abundâncias das espécies e de alguma variação entre anos em vários índices. As variações sazonais ao nível da equitabilidade (que aconteceram no verão), poderão estar relacionadas com a biologia reprodutiva das espécies e com as migrações associadas à reprodução das mesmas, uma vez que os juvenis da maior parte dos peixes que utilizam este estuário como área de viveiro, atingem densidades muito elevadas no estuário na primavera/verão. Verificou-se também um gradiente espacial (provavelmente causado pela salinidade), de montante a jusante, com as riquezas específica e funcional, mais elevadas a jusante e mais baixas a montante enquanto que a equitabilidade das espécies mais baixa a jusante e mais elevada

na zona central do estuário. Relativamente à diversidade filogenética, não foi notório um gradiente espacial, uma vez que existiu uma diversidade filogenética maior a montante e a jusante do estuário e menor na zona central, o que poderá estar relacionado com uma maior diversidade característica das zonas marinhas e dulçaquícolas adjacentes ao estuário do que na área estuarina.

Palavras chave: comunidades de peixes, biodiversidade, Mondego, características funcionais, fatores ambientais.

Resumo alargado

Os estuários são zonas de transição entre o mar e o rio e são caracterizados por serem zonas altamente produtivas. Devido às características destes ecossistemas (alta variabilidade ambiental, nomeadamente diferentes habitats, salinidade e profundidade) os organismos que os frequentam são tolerantes a alterações ambientais. No entanto, as alterações antropogénicas e consequente perda de diversidade que tem vindo a acontecer a nível mundial está a afetar fortemente estes ecossistemas. Os fatores ambientais e antropogénicos que estão associados a esta diminuição da diversidade nos estuários incluem, entre outros, a destruição de habitat, as alterações climáticas, a poluição e a sobre-exploração.

O presente estudo foi realizado no estuário do Mondego, um estuário temperado localizado na costa oeste portuguesa, em que na zona da foz o curso de água se divide, formando a Ilha da Morraceira entre os dois braços do estuário. Este estuário tem sido alvo de várias intervenções antropogénicas, nomeadamente a nível estrutural tendo sido restabelecida a comunicação entre os dois braços do estuário após quase uma década em que esta conexão esteve fechada. Este restabelecimento da conexão entre os dois braços levou a um aumento geral da qualidade da água (que tinha vindo a piorar enquanto a conexão dos dois braços esteve fechada), levando a uma recuperação gradual das comunidades biológicas. Desde então, este estuário tem sido alvo de um intenso programa de monitorização, incluindo amostragens periódicas das comunidades de peixes, não só, mas também, devido ao elevado interesse económico de algumas espécies.

O conceito de biodiversidade engloba várias vertentes (taxonómica, funcional e filogenética), que avaliam propriedades distintas das comunidades. A diversidade taxonómica representa o número de espécies e as suas abundâncias numa comunidade e é uma das medidas mais utilizadas a nível taxonómico. A diversidade funcional mede a distribuição das espécies e as suas abundâncias no espaço funcional ocupado pela comunidade, em que o espaço funcional é definido pelas características funcionais que a comunidade possui. Estas características funcionais são intrínsecas a cada organismo e afetam o seu desempenho na comunidade em que está inserido, influenciando o funcionamento do ecossistema. A diversidade filogenética mede as diferenças evolutivas entre as espécies pertencentes a uma comunidade. Estas três vertentes da biodiversidade estão naturalmente interligadas. Alguns autores têm relatado padrões semelhantes entre riqueza específica e funcional e outros têm destacado a riqueza específica como indicador da diversidade filogenética, mas há também descrições contrárias. Para além disso as diversidades funcionais e filogenéticas também estão relacionadas devido a processos evolutivos uma vez que aumentando a diversidade filogenética também aumenta a diversidade nos indivíduos de uma comunidade, nomeadamente nas suas características funcionais.

Este trabalho teve como principal objetivo avaliar as alterações temporais, sazonais e espaciais em três vertentes da biodiversidade (taxonómica, funcional e filogenética) nomeadamente na comunidade de peixes do estuário do Mondego. Foi utilizada uma série temporal de dados (2003-2013) com informação sobre as abundâncias das espécies de peixes amostradas ao longo de um gradiente salino do estuário do Mondego (5 estações de amostragem). Foram ainda utilizadas seis variáveis para fazer a caracterização ambiental das varias estações de amostragem, nomeadamente a temperatura, salinidade, oxigénio, fluxo de água, precipitação e o índice *North Atlantic Oscilation* (NAO). Cada

espécie de peixe foi classificada taxonomicamente, funcionalmente e filogeneticamente. Ao nível funcional, foram descritas cinco características: preferência de salinidade, mobilidade, dieta, modo de alimentação e comprimento máximo do corpo. Foram ainda caracterizadas as relações filogenéticas entre as espécies, com a utilização de dados referentes a dois genes mitocondriais [16s e *Cytochrome oxidase subunit I* (COI)] - extraídos de duas bases de dados (GenBank e European Nucleotide Archive – ENA) e complementados com análises em laboratório para algumas espécies quando não havia informação disponível. Foram utilizados vários índices de diversidade para cada vertente da biodiversidade (taxonómica – riqueza específica, Shannon-Wiener, Simpson e Pielou; funcional – espécies funcionalmente únicas, riqueza funcional, equitabilidade funcional, divergência funcional, dispersão funcional e índice de Rao; e filogenética – índice de Rao e distância mínima entre pares de espécies) e para uma comparação direta entre as vertentes foi utilizado o índice de Rao. A variabilidade temporal, sazonal e espacial dos índices de diversidade (assim como das variáveis ambientais) foi avaliada através de uma análise de variância com permutações (PERMANOVA). A relação entre os vários índices de diversidade e as suas relações com as variáveis ambientais foi avaliada através de correlações, e foram usadas regressões lineares para avaliar tendências ao longo do período de estudo.

Os resultados obtidos no presente trabalho indicam alguma estabilidade da comunidade de peixes do estuário do Mondego, uma vez que não existiram diminuições na diversidade (taxonómica, funcional e filogenética), com exceção da riqueza funcional que diminuiu ligeiramente. Nos últimos anos tem sido registado um aumento da salinidade neste estuário e uma diminuição do número de espécies de água doce (o que poderá ser a causa da diminuição da riqueza funcional) para além de um aumento gradual no número de espécies marinhas. Na década abrangida pelo presente estudo, o ganho de umas espécies (marinhas) parece ter contrabalançado a perda de outras (dulçaquícolas), ainda que com a perda de algum espaço funcional. Adicionalmente, a diversidade filogenética não diminuiu o que poderá ter contribuído para a manutenção da diversidade taxonómica e funcional. Finalmente, os organismos que habitam os estuários são naturalmente tolerantes a variações ambientais o que pode ter contribuído de alguma forma para esta estabilidade.

Para além disto, a nível sazonal não foram verificadas alterações na diversidade dos peixes do estuário com exceção da equitabilidade. Ao nível da equitabilidade o verão foi a estação que se destacou com os valores mais baixos, o que poderá ser explicado pela biologia reprodutiva das espécies que utilizam o estuário como área de viveiro, em que os seus juvenis atingem abundâncias maiores nas estações primavera/verão, reduzindo a equitabilidade. E ao nível da distribuição espacial os resultados apontam para um gradiente de diversidade crescente de montante para jusante (a nível de riqueza específica e funcional), mas menor equitabilidade (algumas espécies a representarem a maior parte da comunidade, enquanto outras se encontram representadas por poucos indivíduos) na zona mais salina onde a riqueza específica e funcional é maior. Relativamente à diversidade filogenética, também não se verificou um gradiente espacial. Os nossos resultados apontam para uma maior diversidade nas áreas mais a montante e mais a jusante e uma menor diversidade na zona central do estuário, que poderá estar relacionado com uma maior diversidade que existe nas zonas marinhas e dulçaquícolas adjacentes aos estuários quando comparada com as zonas estuarinas.

Este trabalho revelou novas perspetivas acerca da relação dos índices de diversidade e vem acrescentar conhecimento sobre a dinâmica da biodiversidade das comunidades de peixes estuarinas. Contudo, estudos futuros deverão aprofundar a influência de pressões antropogénicas e testar o efeito da utilização de outro conjunto de características funcionais. Apesar da estabilidade da comunidade de peixes do estuário do Mondego, existe uma constante e crescente pressão, quer antropogénica quer ambiental que poderá levar a uma perda de diversidade. Assim sendo, um maior conhecimento sobre as

alterações na comunidade de peixes será útil para a definição de medidas de gestão e conservação tendo em vista o controlo da perda de diversidade neste estuário e dos serviços de ecossistema que este fornece.

Palavras chave: comunidades de peixes, biodiversidade, Mondego, características funcionais, fatores ambientais.

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CHAPTER 1

General introduction

General introduction

Estuarine areas are transitional ecosystems, which are characterized for its high productivity (Costanza *et al.* 1997). These highly productive transitional zones are very important to many fish species that use these locations as nursery grounds or migration routes and are also important to estuarine residents (Elliott & Dewailly 1995; Beck *et al.* 2001; Elliott *et al.* 2007; Dolbeth *et al.* 2008; Franco *et al.* 2008; Vasconcelos *et al.* 2009; Elliott & Whitfield 2011). Moreover, estuarine habitats such as seagrass and saltmarshes are especially valuable in providing both refuge from predators and food (due to its high productivity) to the fish assemblages (Beck *et al.* 2001; Cabral *et al.* 2007; Dolbeth *et al.* 2008). Estuaries are known for their natural environmental variability, with different habitats, salinities and depths (Maes *et al.* 2004; Elliott & Quintino 2007; Dolbeth *et al.* 2008; Courrat *et al.* 2009). Estuarine organisms are known to be very tolerant to environmental variations and are consequently resilient to changes and so climate and anthropogenic pressures in these systems could potentially have a smaller impact than in other aquatic systems (Elliott & Whitfield 2011). However, despite their ecological importance, estuarine areas are among the most threatened ecosystems, due to climate changes (Martinho *et al.* 2007) and anthropogenic pressures (Vasconcelos *et al.* 2007). Moreover, with the global tendency of biodiversity loss, estuarine systems are experiencing great losses in populations and entire functional groups (Lotze *et al.* 2006; Worm *et al.* 2006).

The Mondego estuary is a warm-temperate system located in the western coast of Portugal (Baptista *et al.* 2015). This estuary has been subjected to several anthropogenic interventions, namely the closing of the connection between the two arms of the estuary between 1990 and 1997, which resulted in water circulation in the south arm becoming dependent from precipitation and freshwater input from the Pranto River (Neto *et al.* 2010). Moreover, Mondego estuary is also exposed to other anthropogenic effects such as dredging, shipping activities and sewage discharge (Vasconcelos *et al.* 2007). These anthropogenic effects led to an increase in eutrophication and degradation of water quality (Verdelhos *et al.* 2014) and to increasing salinity, resulting in the decrease in the number of freshwater species in this estuary (Leitão *et al.* 2007; Nyitrai *et al.* 2012). In 1998, a management plan was implemented in the Mondego estuary, that re-established the connection between the two arms, resulting in a gradual ecological recovery (Lillebø *et al.* 2005; Cardoso *et al.* 2007; Cardoso *et al.* 2010; Dolbeth *et al.* 2011; Verdelhos *et al.* 2014). Since then, this estuary has been the object of a long-term monitoring program and of several complementary studies on its biological communities (e.g., Leitão *et al.* 2007; Dolbeth *et al.* 2008; França *et al.* 2009; Baptista *et al.* 2010; Martinho *et al.* 2010; Primo *et al.* 2013;

Baptista *et al.* 2015). The fish assemblage in the Mondego estuary is mainly dominated by the estuarine residents *Pomatoschistus microps* and *Pomatoschistus minutus*, by juveniles of the marine migrants *Dicentrarchus labrax, Solea solea* and *Platichthys flesus*, and also of *Diplodus vulgaris* (Martinho *et al.* 2007). Furthermore, there are many species with high economical value in this estuary, namely *Anguilla anguila, Chelon labrosus, Dicentrarchus labrax, Diplodus vulgaris, Liza ramada, Platichthys flesus, Sardina pilchardus* and *Solea solea* (Vasconcelos *et al.* 2009; Baptista *et al.* 2013).

Biodiversity is a wide concept and is a useful indicator of ecological quality (Bechtel & Copeland 1970; Barbosa *et al.* 2001). Most ecological studies on biodiversity have been focused on taxonomic approaches, mainly using species richness or other indices that also account for abundance such has Shannon-Wiener or Simpson indices (e.g., Grubb 1977; Currie & Paquin 1987; Zobel 1997; Oliveira & Amaral 2004). More recently, there has been an increase in studies which focus on other facets of diversity, namely functional (e.g., Mason *et al.* 2007; Mouillot *et al.* 2013) and phylogenetic (e.g., Nixon & Wheeler 1992; Kellar *et al.* 2015) and even on two or three of these facets simultaneously (e.g., Devictor *et al.* 2010; Villéger *et al.* 2010; Flynn *et al.* 2011; Meynard *et al.* 2011; Baptista *et al.* 2015) . The use of only one approach, like for example the taxonomic, is unlikely to provide enough information about the communities and can be unable to reveal changes that may be happening (e.g., Villéger *et al.* 2010).

Effects of environmental changes on the fish assemblages are well documented worldwide (e.g., Thiel *et al.* 1995; Marshall & Elliott 1998; Meynecke *et al.* 2006), and have also been documented in the Mondego estuary (e.g., Martinho *et al.* 2007; Baptista *et al.* 2010; Martinho *et al.* 2010; Nyitrai *et al.* 2012). Effects of changes in river runoff (Livingston 1997), temperature (Elliott & Whitfield 2011) and salinity (Elliott & Whitfield 2011; França *et al.* 2011; Baptista *et al.* 2015) stand out as important features that structure fish assemblages (Thiel *et al.* 1995). For example, the increase in temperature over recent years has been linked to the consequent increase of occurrence of warm water species along the Portuguese coast (Costa *et al.* 2014), whilst decrease in river runoff has been documented to increase species richness, namely the increase in the richness of herbivore species as a result of higher light penetration (Livingston 1997) and in the abundance and richness of marine species (Whitfield & Harrison 2003). Moreover, in estuaries, salinity plays a major role in the composition of the fish assemblages, creating a spatial longitudinal gradient inside the estuary, with different species in different areas with different salinities, for example marine species associated to lower more saline areas and catadromous and freshwater species mainly present in upper less saline areas as is observed in the Mondego (Leitão *et al.* 2007).

The main goal of this study was to investigate changes in taxonomic, functional and phylogenetic diversity of the fish assemblage in the Mondego estuary. Using a time series (2003-2013)

we characterized spatial and temporal changes in diversity (taxonomic, functional and phylogenetic) of the fish assemblage of the Mondego estuary which could be related with environmental changes.

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CHAPTER 2

Changes in taxonomic, functional and phylogenetic diversity of the fish assemblage in a temperate estuary

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Changes in taxonomic, functional and phylogenetic diversity of the fish assemblage in a temperate estuary

Abstract

Worldwide, rapid biodiversity loss has been widely documented including in estuarine ecosystems. We assessed spatial, seasonal and inter-annual changes (throughout a decade 2003-2013) in the fish assemblage of the Mondego estuary, namely in biodiversity (taxonomic, functional and phylogenetic) and examined relationships with environmental variables. Meanwhile we used Rao's Quadratic Entropy index to compare the three facets of biodiversity. Our results about the environmental effects are corroborated by previous studies, where salinity, temperature and river runoff were the variables that most influence the fish assemblage. Despite some inter-annual variability, results enhanced the temporal stability of the fish assemblage, with only functional richness showing a significant slight decline through the sampling period, and seasonal variations in taxonomic evenness (summer highlighted with low evenness). Decrease in functional richness may be related with the disappearance of freshwater species due to an increase in salinity in the last years, while seasonal patterns in taxonomic may be explained by reproductive biology of fishes since summer is the season where some of the most abundant species attain higher juvenile densities, resulting in a decrease of evenness. However, evenness indices generally followed different patterns than richness indices, namely where richness was higher (site M which is near to the sea) evenness indices was lower. Results also suggest a spatial gradient, with species and functional richness increasing from upstream to downstream, while phylogenetic diversity was higher in both upstream and downstream and lower in middle estuary. Regarding the three Rao indices (taxonomic, functional and phylogenetic), results are in agreement with several studies that report relationships between the three facets of diversity. Results bring new perspectives regarding the variation of diversity indices and also strengthens previous knowledge about estuarine fish assemblages.

Key-words: fish assemblages, biodiversity, Mondego, functional traits, environmental effects

Introduction

Estuaries are highly productive and valuable transition zones between rivers and sea (Costanza *et al.* 1997). The transitional nature of estuaries associated with their low depth, muddy grounds and salinity variation increase the diversity of habitats and food availability which makes estuarine areas important nursery and refuge areas for a variety of fish species, beyond allowing the migration between marine and freshwater ecosystems (Whitfield 1990; Elliott & Dewailly 1995; Potter & Hyndes 1999; Franco *et al.* 2008). Losses of estuarine habitats can lead to a decrease in biodiversity with several consequences to Human wellbeing, for instance decrease of valuable species for fisheries (Connolly 1994; Jenkins *et al.* 1997).

Biodiversity is a wide concept that can be defined by "the variety of life, at all levels of organization, classified both by evolutionary (phylogenetic) and ecological (functional) criteria"

(Colwell 2009). And it represents a valuable natural resource at economic and cultural levels. Biodiversity is also a useful ecological indicator (Bechtel & Copeland 1970; Barbosa *et al.* 2001), and is commonly used in ecological monitoring. Worldwide, biodiversity is decreasing due to habitat destruction, pollution, overexploitation of resources, climate change and disturbances on the biological, geological and chemical properties of ecosystems (Jackson *et al.* 2001; Dulvy *et al.* 2003; Pandolfi *et al.* 2003; Worm *et al.* 2005; Lotze *et al.* 2006). In fact, at a global scale more and more species are increasingly under threat (Butchart *et al.* 2005) and the populations of several vulnerable species are declining (Loh *et al.* 2005), and ecosystems like estuaries (Lotze *et al.* 2006) are experiencing big losses in populations, number of species and even functional groups (Worm *et al.* 2006). Several studies showed that biodiversity loss causes instability in ecosystems (Frank & McNaughton 1991; McNaughton 1994; Tilman 1996; Tilman & Downing 1996; McGrady-Steed *et al.* 1997; Naeem & Li 1997; Petchey *et al.* 1999) and affect ecosystem services. However, ecosystem stability can't necessarily be examined at species composition level, since an increase in species can for example increase competition (May 1973; Lehman & Tilman 2000). Actions towards preserving biodiversity should take into consideration taxonomic, evolutionary (i.e. phylogenetic diversity) and ecological criteria (i.e. functional diversity)(Colwell 2009). There has been an increase of studies using these three dimensions of biodiversity providing complementary information about community structure (*i.e.* taxonomic, functional and phylogenetic), instead of simply using the most used approach - taxonomic diversity (Díaz *et al.* 2007; Graham & Fine 2008; Devictor *et al.* 2010; Meynard *et al.* 2011; Safi *et al.* 2011). Phylogenetic diversity can reflect the evolutionary history of a community (Webb *et al.* 2002) and functional diversity is considered an adequate way of explaining ecosystem functioning (Hooper *et al.* 2005; Mouchet *et al.* 2010).

Taxonomic diversity represents the number of species and their abundances within a community, and species richness is one of the most important taxonomic diversity metrics, since it is the base of a great variety of ecology models (Macarthur & Wilson 1967; Connell 1978; Stevens 1989; Gotelli & Colwell 2001) and is crucial to understand community diversity (Gotelli & Colwell 2001). However, this metrics relies on the assumption that all species are equal with only their relative abundances differing (Magurran 2004). Therefore, different approaches need to be used, since relative abundance is not the only aspect differing between species in a community (Mouchet *et al.* 2010).

Functional diversity measures the distribution of species and their abundances within the functional space occupied by the community (Mouillot *et al.* 2013). Functional traits are the characteristics of an organism that affect its performance in the community (Tilman 2001; Mouillot *et al.* 2013). Functional diversity measures have been used in many recent studies (Kahmen & Poschlod 2004; Poff *et al.* 2006; Mason *et al.* 2007; Nyitrai *et al.* 2012; Mouillot *et al.* 2013). Functional traits influence ecosystem functioning (Tilman 2001; Mouillot *et al.* 2013), its productivity (Tilman *et al.* 1997; Hooper & Dukes 2004; Petchey *et al.* 2004; Hooper *et al.* 2005; Villéger *et al.* 2008) and its resilience to perturbations or invasions (Dukes 2001; Bellwood *et al.* 2004; Folke *et al.* 2004; Villéger *et al.* 2008).

The increase in taxonomic diversity can involve an increase of functional diversity, since higher number of species in a community increases the probability that more functional traits are present, as shown by Baptista *et al.* (2015) that reported the same patterns for species and functional richness in the Mondego estuary. However for example Villéger *et al.* (2010) reported an increase in taxonomic diversity in a fish community after habitat degradation, while functional diversity experienced big losses, highlighting that taxonomic diversity approaches can provide incomplete information about diversity changes in a community and that conservation efforts shouldn't focus only on species richness, but also on other characteristics of the community, like its functional traits. Moreover, when species from different locations differ (due to biogeography, habitat and environmental characteristics) direct

ecological comparisons based on taxonomy have limited interest. A functional approach based on the role of species in the ecosystem (functional traits) allows the comparison of the communities (McGill *et al.* 2006) and a way of quantifying the role of each species in the ecosystem.

Phylogenetic diversity reflects the accumulated evolutionary history of a community (Forest *et al.* 2007) and so can be related to the community's capacity to adapt, *i.e.* generate new evolutionary solutions when facing changes (Forest *et al.* 2007; Faith 2008). Phylogenetic diversity indices measure the length of evolutionary differences between the members of a community (Vane-Wright *et al.* 1991; Faith 1992). Therefore, high phylogenetic diversity may increase the community species' diversification increasing the probability of its survival (Forest *et al.* 2007).

Phylogenetic diversity is also linked to taxonomic and functional diversity, with some studies for example indicating species richness as an indicator of phylogenetic diversity (Barker 2002; Rodrigues & Gaston 2002; Schipper *et al.* 2008; Devictor *et al.* 2010), even though others showing no relationship between them (Forest *et al.* 2007; Devictor *et al.* 2010). Phylogenetic diversity seems to be more related to functional diversity (than taxonomic diversity) due to evolutionary processes (Webb *et al.* 2002), based on the assumption that phylogenetic diversification in a community will generate diversification in the individuals of that community, namely in their characteristics (trait diversification) (Flynn *et al.* 2011). Consequently the maintenance of taxonomic, and also phylogenetic diversity could imply a maintenance (at least partially) of functional diversity (Forest *et al.* 2007; Cadotte *et al.* 2009) and therefore of the functioning of the ecosystem (Díaz *et al.* 2007; Cadotte *et al.* 2009).

In this study, we combine the information provided by three facets of biodiversity (i.e. taxonomic, functional and phylogenetic) to assess their spatial (within a salinity gradient within Mondego estuary) and temporal variation (along 10 years). Specifically, the main goals were to investigate if: (1) taxonomic, functional and phylogenetic diversity have the same tendencies along environmental gradients; (1) if the three biodiversity facets have the same trends through time (between years and within years - seasonally). To do so we used a time series database with periodic samplings of the fish assemblage in the Mondego estuary over ten years (2003-2013). The use of time series for biodiversity studies is an approach already used by several authors (Loh *et al.* 2005; Shimadzu *et al.* 2013; Dornelas *et al.* 2014). But the combined use of these three facets of biodiversity has been recently developed, and has had limited advancement in fish assemblages especially using time series.

Materials and Methods

Study area

The Mondego estuary (40°08'N, 8°50'W) is a warm-temperate system located in the North East Atlantic – Portugal, with 8.6 km^2 of total area (Baptista *et al.* 2015). It comprises two arms separated at 7km from shore and joined again near the mouth, creating the Morraceira Island between the arms. The north arm is deeper (5-10 m during high tide) than the south arm (2-4 m during high tide) (Flindt *et al.* 1997). The south arm is largely silted up in the upstream areas, causing the water to flow mainly through the north arm, which makes the water circulation on the south arm mostly dependent on tides and on small freshwater inputs from the Pranto River. These freshwater inputs are controlled by a sluice, depending on the needs in the rice fields along the Mondego agricultural valley (Baptista *et al.* 2013). There are four main habitats in the estuary: subtidal soft substratum, intertidal soft substratum, tidal freshwater and saltmarsh (França *et al.* 2009). The downstream areas of the south arm have an important seagrass community of *Zostera noltii (Dolbeth et al. 2008).*

The fish assemblage of the Mondego estuary is dominated by estuarine residents *Pomatoschistus microps* and *Pomatoschistus minutus, by juveniles of* species that use the estuary as nursery grounds *Dicentrarchus labrax, Solea solea* and *Platichthys flesus,* and of marine migrant species *Diplodus vulgaris* (Martinho *et al.* 2007b). Moreover, many species have high socio-economic importance, namely: *Anguilla anguilla, Chelon labrosus, Dicentrarchus labrax, Diplodus vulgaris, Liza ramada, Platichthys flesus, Sardina pilchardus* and *Solea solea* (Vasconcelos *et al.* 2009; Baptista *et al.* 2013).

The Mondego estuary is exposed to several anthropogenic effects from activities such as dredging and shipping in the north arm and sewage and discharge of nutrients from agriculture and fish farms in upstream areas of the south arm (Vasconcelos *et al.* 2007; Martinho *et al.* 2015). To mitigate the impacts of the anthropogenic pressures in the estuary and improve water quality, several interventions were implemented namely the re-establishment of the communication between the two arms in 1998 and the enlargement of the connection between the two arms in 2006 (Neto *et al.* 2010; Nyitrai *et al.* 2013). These measures improved water circulation in the south arm (Neto *et al.* 2010; Nyitrai *et al.* 2013). In addition, increase of temperature and occurrence of weather extremes such as floods and droughts in the last fifteen years and consequent changes in salinity have been linked to changes in the fish assemblage (Martinho *et al.* 2007b; Martinho *et al.* 2010; Baptista *et al.* 2015).

Sampling procedures

A long-term monitoring program targeting the fish assemblage has been implemented in the Mondego estuary from 2003 to the present. Sampling was performed approximately monthly between June 2003 and June 2013 at five sampling sites (M, N1, N2, S1, and S2) (Fig. 2.1). Site M, the most downstream, is subjected to frequent dredging and is the deepest (8.7 ± 1.2 m). Sites N1 and N2 are located in the North arm and have regular freshwater flow: site N1 in the middle reaches of the estuary, and N2 is the most upstream site and has low salinity. Sites S1 and S2 are located in the south arm and are shallower: S1 (2.3 ± 0.4m) is located in the middle reaches near the *Zostera noltii* bed, while S2 (2.4 ± 1.0 m) is located upstream near the Pranto river sluice. Fishing took place during the night, using a 2-m beam trawl with one tickler chain and 5 mm mesh size in the cod end. At each sampling site, three replicate hauls were towed for an average of 3 min each, covering an area of at least 500 m^2 for each replicate. All fish were preserved in iceboxes and transported to the lab where they were counted, identified, measured (total length, with 1 mm precision) and weighted (wet weight).

Several environmental variables were also measured to characterize the sampling sites. Namely, during fish campaigns before each tow, water temperature (°C), salinity, dissolved oxygen (%) were measured at the bottom. Monthly precipitation (mm) and freshwater runoff (dam^3) were obtained from Instituto Nacional da Água (INAG) stations Casal do Rato 13D/04UG and Açude Ponte Coimbra 12G/01A near the city of Coimbra located 40 km upstream, respectively (http://snirh.pt). North Atlantic Oscillation Index (NAO) was obtained from the National Oceanic Administration Atmospheric website (http://www.noaa.gov). The environmental data were obtained for each one of the 5 sampling sites and we used in this study the mean value of each environmental variable across all sites.

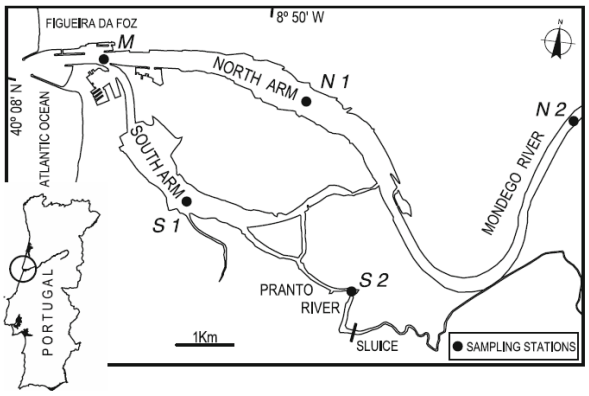

Figure 2.1 – Map of the Mondego estuary, with the location of the sampling stations.

Fishes functional traits

The species of the fish assemblage in the Mondego estuary sampled during the study period (total=43 species) were classified based on five functional traits: salinity preference, mobility (adapted from Henriques *et al.* 2013), diet (adapted from Elliott *et al.* 2007), feeding mode and maximum body length (For more information about definitions and categories of each functional trait see Tables 1 - Appendix 1). Information about the functional traits of species was collected from the literature and publicly available databases such as Fish-Base, Marine Species Identification Portal, Marine Life Information Network (MarLIN), Encyclopedia Of Life (EOL), IUCN, Sea Around us Project and AnAge: The Animal Ageing and Longevity Database. Species diet and feeding mode were classified considering their predominant life stage/s within estuaries.

Fishes phylogenetic tree

We also characterized the phylogenetic relationships between species of the fish assemblage of the Mondego estuary. We selected and extracted two mitochondrial genes that are frequently used and were available in Genbank and European Nucleotide Archive (ENA) – 16s and Cytochrome oxidase subunit I (COI) (Simons & Mayden 1998; Roudbar *et al.* 2016). However, some sequences weren't available neither in GenBank or ENA and were newly sequenced. Total genomic DNA was extracted from fin clips preserved in ethanol using REDExtract-N-Amp Tissue PCR kits (Sigma-Aldrich) following the manufacturer's instructions. Both 16s and COI were amplified using the primers and PCR conditions shown in Table 2 in Appendix 1). PCR products were purified and sequenced, using forward primers, at STABVIDA (Portugal). It was not possible to obtain a COI gene sequence for *Parablennius gattorugine* neither in GenBank, ENA or newly sequenced that we could assemble to the others. Therefore, and since there were no other Blenniidae in our samples, we used a COI gene sequence of *Parablennius rouxi,* which also occurs in this geographical area (Almada *et al.* 2005). The complete dataset included eight newly obtained sequences and seventy-eight sequences retrieved from Genbank and ENA (Table 2.1). All sequences were trimmed at the 3' and 5' ends so they had the same length for all the individuals sampled, after alignment and editing using CodonCode Aligner v4.0.4 (CodonCode Corp., USA). PAUP software package V.4.0 (Swofford 2003) was used to estimate the corrected pairwise distances between species which were used subsequent analysis. PAUP was also used to reconstruct phylogenetic relationships between species by maximum parsimony (MP; heuristic searches, 10 random stepwise additions) and neighbour-joining (NJ). Branch support was tested by bootstrap analysis, with 1000 resamplings.

Table 2.1 – Accession numbers of the 16S and COI gene sequences obtained from Genbank and ENA databases for all fish species caught in the Mondego estuary.

	Mitochondrial genes	
Species	16s	COI
Ammodytes tobianus	AY141450	*
Anguilla anguilla	AB021749	KJ768203
Aphia minuta	KF415305	KJ204702
Arnoglossus laterna	AY359653	JQ774968
Atherina boyeri	HM855100	KJ552752
Atherina presbyter	HM855086	KF929634
Buglossidium luteum	JN312474	KJ128718
Callionymus lyra	KJ128720	JQ774796
Carassius auratus	KM657137	KM286501
Chelidonichthys lucerna	KJ128733	JQ775001
Chelon labrosus	KF375095	JQ060484
Ciliata mustela	KC980956	KJ204807
Conger conger	*	JQ775007
Dicentrarchus labrax	HQ731430	KJ205274
Dicologlossa hexophthalma	AB125253	EU513729
Diplodus vulgaris	GQ485269	JX192141
Echiichthys vipera	*	JQ775014
Engraulis encrasicolus	JN103418	KF929837
Gaidropsarus mediterraneus	KC980953	JQ774626
Gambusia holbrooki	*	KJ553402
Gobius niger	KJ128784	KJ768236
Liza aurata	KF375034	KC500832
Liza ramada	KF375038	JQ775059
Luciobarbus bocagei	*	KJ554052
Mugil cephalus	KF375051	KC500952
Mullus surmuletus	KJ128837	JQ774875
Nerophis lumbriciformis	*	*
Parablennius gattorugine	DQ160200	AJ872148 *[1]
Pegusa lascaris	AF112849	KJ205298
Platichthys flesus	AF113180	KJ768280
Pomatoschistus microps	KJ128869	KJ768285
Pomatoschistus minutus	KJ128870	KM077866
Sardina pilchardus	FR849604	KJ205157
Scophthalmus rhombus	KJ128899	KJ768304
Solea senegalensis	AB125246	KF369185
Solea solea	JQ939100	JQ774922
Sparus aurata	AF247432	KJ012434
Spondyliosoma cantharus	AJ247280	JQ775133
Symphodus bailloni	AY092037	GQ341601
Syngnathus abaster	AF355010	KJ554618
Syngnathus acus	KJ128916	GQ502180
Syngnathus typhle	KJ128918	KJ128634
Trisopterus luscus	KC980929	JQ775158

* There were no data in GenBank and ENA available for this species.

*[1] In the absence of COI sequence for *Parablennius gattorugine* we used a COI sequence of *Parablennius rouxi*.

Data analysis

In the subsequent analyses, a sample consisted of the mean density of species sampled in a given site and date.

Diversity indices – Taxonomic diversity

For each sample in the database we estimated taxonomic diversity through the number of species (*S*), Simpson, Shannon-Wiener indices and Pielou's evenness index (R Development Core Team, 2016).

Simpson's diversity index (*D*) measures the probability of two randomly selected individuals in a sample belonging to the same species, meaning that 0 represents infinite diversity and 1 no diversity (Scheiner 2012). Simpson index can be calculated as:

$$D = \sum_{i=1}^{R} p_i^2 \tag{2.1}$$

where p_i are the proportional abundances of the types of interest, i.e. the abundances of our species in the community. We used *1-D* Simpson index so that a higher index value represents higher diversity.

Shannon-Wiener diversity index ($D_{Shannon}$) quantifies the uncertainty in the prediction of which species a randomly selected individual belongs to (Scheiner 2012). Higher values mean that it is harder to predict the species of a randomly selected individual and therefore that the community has more species, meaning the diversity is higher. It can be calculated as:

$$D_{Shannon} = -\sum_{i=1}^{R} p_i \ln(p_i) \tag{2.2}$$

where p_i is the proportion of individuals belonging to the i^{th} species in the community.

Pielou index quantifies how equal are the abundances of the species in a community (Pielou 1966). The rationale is that since diversity is a mixture of richness and evenness, if we remove richness than we should obtain evenness (Scheiner 2012). Pielou's evenness is calculated by:

$$\text{Pielou} = \frac{D_{Shannon}}{\ln(S)} \tag{2.3}$$

where *D* is the Shannon-Wiener diversity and *S* the number of species (Scheiner 2012).

Diversity indices – Functional diversity

Several functional diversity indices (i.e. functionally singular species, functional richness, functional evenness, functional divergence, functional dispersion and Rao's Quadratic entropy) were determined (Laliberté & Legendre 2010; Laliberté *et al.* 2015, R Development Core Team, 2016).

Functionally singular species (*FSing*) are the species with a unique combination of functional traits and functional richness (*FRic*) is the amount of functional space filled by a community (Villéger *et al.* 2008) and is measured as the convex hull volume.

Functional evenness (*FEve*) describes how regularly are the species abundances distributed in a community, with high values meaning that the species and their abundances are well distributed, and low values that the distribution is not regular (Villéger *et al.* 2008).

$$FEve = \frac{\sum_{l=1}^{S-1} min\left(PEW_l, \frac{1}{S-1}\right) - \frac{1}{S-1}}{1 - \frac{1}{S-1}} \tag{2.4}$$

where PEW is the partial weighted evenness and *S* is the number of species (Villéger *et al.* 2008).

Functional divergence (*FDiv*) represents how abundance is distributed among the functional space occupied by a community (Villéger *et al.* 2008). FDiv is calculated by:

$$FDiv = \frac{\Delta \mathrm{d} + \overline{\mathrm{dG}}}{\Delta |\mathrm{d}| + \overline{\mathrm{dG}}} \quad (2.5)$$

where Δd is the sum of abundance-weighted deviances, $\overline{dG}$ is the mean distance of species to the centre of gravity and $\Delta|d|$ is the absolute abundance-weighted deviances (Villéger *et al.* 2008). Low values mean that the most abundant species are functionally closer to the centroid. Therefore, if those species stray from the centroid, functional divergence values will be higher.

Functional dispersion (*FDis*) is the mean distance of each species to the centroid, measured with all species in the community (Laliberté & Legendre 2010). It counts for relative abundances defining this centroid based in the most abundant species and then computing a weighted average distance of each species to the centroid (Laliberté & Legendre 2010), using species abundances as weights. Centroid *c* is computed as:

$$c = ci = \frac{\sum a_j x_{ij}}{\sum aj} \quad (2.6)$$

where *c* is the weighted centroid, *aj* the abundance of *j* species, and *xij* the attribute of *j* species for trait *i* (Laliberté & Legendre 2010). Functional dispersion is now computed as:

$$FDis = \frac{\sum a_j z_j}{\sum a_j} \quad (2.7)$$

where a_j is the abundance of *j* species and z_j is the distance of *j* species to the centroid *c* (Laliberté & Legendre 2010).

Like functional dispersion, Rao's Quadratic entropy (RaoQ) is a dispersion index and for functional diversity (*FRaoQ*) it measures the functional differences between all pairwise species (Botta-Dukát 2005). *FRaoQ* is calculated by:

$$\mathrm{FRaoQ} = \sum d_{ij} p_i p_j \quad (2.8)$$

where d_{ij} is the functional distance between two species (*i* and *j*), and p_i and p_j are species relative abundances.

Diversity indices – Phylogenetic diversity

To assess the phylogenetic diversity in our communities, we determined Rao's Quadratic entropy for phylogenetic diversity (*PRaoQ*) by using a distance-distance matrix (Debastiani & Pillar 2012, R Development Core Team, 2016).

$$\mathrm{PRaoQ} = \sum d_{ij} p_i p_j \quad (2.9)$$

Where d_{ij} represents the phylogenetic distances between two species (*i* and *j*) and p_i and p_j are species relative abundances (Meynard *et al.* 2011).

We also calculated the mean pairwise distance between species in our communities (Kembel *et al.* 2010, R Development Core Team, 2016). Mean pairwise distance represents the average evolutionary distance between all pairwise species in our community, where the higher the value, the higher number of species with above-average branch lengths, indicating that phylogenetic diversity is higher (Webb 2000).

Statistical analysis

Pearson correlations between all environmental variables and among all estimated diversity indices (i.e. between taxonomic indices, between functional indices and between phylogenetic indices, and between the three RaoQ) were performed to assess their relationships (Revelle 2015, R Development Core Team, 2016). Moreover, linear regressions were also used to investigate if there was an increasing or decreasing tendency through time in each environmental variable and also in each diversity index. Linear regressions were also used between each diversity index and each environmental factor to assess

the influence of each environmental factor on the diversity indices. Factorial Permutational Analysis of Variance (PERMANOVA) followed by post-hoc tests for significant relationships, was conducted to investigate differences in these diversity indices between sites, seasons and years (using Primer 6 software) including interactions. PERMANOVA uses permutations to test the simultaneous response of variables to factors. Hence ANOVA assumptions do not apply i.e. that the errors are independent, that they are normally distributed with a mean of zero and have a common variance, and that they are added to the treatment effects (Anderson *et al.* 2008). PERMANOVA analyses were also conducted for environmental data to search for variations between sites, years and seasons including interactions.

To directly compare taxonomic, functional and phylogenetic diversity we used the Rao's Quadratic Entropy index, which allow us to make a direct comparison between all three types of diversity measures (Jost 2007; Ricotta & Szeidl 2009; De Bello *et al.* 2010). In the case of taxonomic diversity, Simpson's diversity index becomes equivalent to the RaoQ index (Meynard *et al.* 2011), so we used the Simpson index.

Results

Environmental features

Correlations between environmental variables were generally significant (Table 2.2), with the highest correlations observed between runoff and precipitation (R=0.38) followed by a negative correlation between temperature and oxygen (R=-0.36) (Table 2.2). Only salinity (R^2=0.01255), oxygen (R^2=0.008615) and NAO index (R^2=0.01739) showed significant linear variation (evaluated with linear regressions) throughout sampled years, with both oxygen and NAO index decreasing through time, while salinity increased.

Table 2.2 –Pearson correlation values (p-value <0.05) between environmental variables of the sampled sites within Mondego estuary. ns – correlation was not significant (p-value >0.05).

	Salinity	Temperature	Oxygen	River runoff	Precipitation	NAO
Salinity						
Temperature	ns					
Oxygen	0.227	-0.356				
River runoff	-0.325	-0.377	0.175			
Precipitation	-0.247	-0.324	ns	0.378		
NAO	ns	-0.121	ns	-0.127	-0.158	

Regarding the PERMANOVA results, temperature showed significant differences between sampling sites, seasons and years, with site M the most different site with low values, summer was the most different season with high values and 2005 the most different year with high values (Table 1 in Appendix 2).

In addition, temperature had two significant interactions: 1) site x season, with autumn being the season with less variations among sampling sites, and all sampling sites showing variations between seasons (especially between Summer-Winter, Summer-Spring, Autumn-Winter and Winter-Spring); and 2) season x year, with all seasons showing several differences between years and 2007 with no variations between seasons (Table 1 in Appendix 2).

Both salinity and oxygen varied between sampling sites, seasons and years - sites M and S1 with high values and S2 with low values the most different, summer in salinity (with high values) and summer and autumn (with low values) in oxygen the most different seasons and years 2003 (low values), 2004 (low values) and 2005 (high values) in salinity and 2003 (high values) and 2005 (high values) in oxygen the most different years (Tables 2 and 3 in Appendix 2). Both factors also had a significant season x year interaction, with all seasons showing several differences between years for both indices, while year 2003 for salinity and years 2004, 2008 and 2012 for oxygen showed no significant variations between seasons (Tables 2 and 3 in Appendix 2).

Both river runoff and precipitation showed variations between seasons and years, with summer the most different season (with low values) and river runoff in 2003 (high values), 2004 (high values), 2005 (low values), 2007 (low values) and 2010 (high values) the most different years. The year of 2005 stood out as the most different year regarding precipitation, with low values. In addition, a significant season x year interaction was also observed between these two parameters – with all seasons showing strong differences between years, with summer being the season with more pairwise results while 2008 showed no differences between seasons for river runoff and all years showed significant variations between seasons in precipitation (Tables 4 and 5 in Appendix 2).

NAO index had a significant season x year interaction, with all years showing variations among seasons and with all seasons showing variations between sampled years, and summer being the season with more pairwise results (Table 6 in Appendix 2).

Table 2.3 – Mean values and standard deviation of the six environmental variables [temperature, salinity, oxygen, river runoff, precipitation and North Atlantic Oscillation (NAO) index] in each site, season and year.

Factor		Temperature		Salinity		Oxygen		River runoff		Precipitation		NAO index	
Level		Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
Site	M	15.39	2.48	30.74	5.68	95.50	9.28	108540.16	163673.93	52.39	51.12	-0.20	1.09
	N1	16.41	3.36	24.37	7.11	90.27	6.72	95356.84	141370.24	48.93	50.45	-0.22	1.11
	N2	17.40	4.69	9.98	9.05	85.61	10.22	98702.64	144577.99	47.66	49.93	-0.22	1.11
	S1	16.01	2.91	31.30	4.92	94.43	7.30	111703.89	169580.05	50.48	47.76	-0.21	1.08
	S2	18.93	5.02	24.86	6.85	86.75	9.57	112332.32	167583.53	51.52	50.92	-0.23	1.09
Season	Autumn	16.71	3.14	24.03	11.19	86.90	9.09	93351.87	146120.98	87.70	70.01	-0.11	0.86
	Spring	17.34	2.95	24.32	10.22	92.86	9.20	77919.04	59149.41	42.15	23.61	0.04	1.29
	Summer	20.02	3.00	26.72	8.58	88.40	9.53	37887.24	10803.77	15.41	14.46	-0.57	1.07
	Winter	11.87	2.10	21.57	10.41	95.10	7.87	252289.36	251426.44	62.36	38.04	-0.17	0.97
Year	2003	18.36	3.93	20.50	11.87	90.81	12.04	147080.00	168519.80	68.80	80.29	0.11	0.49
	2004	15.51	3.99	21.10	10.95	91.86	9.96	115799.03	93283.68	45.14	16.75	0.05	0.79
	2005	16.11	4.53	25.93	9.85	93.50	10.68	45429.12	60067.84	41.40	34.62	-0.41	1.06
	2006	17.72	4.14	22.01	10.70	89.59	9.68	139313.15	182651.38	75.55	59.85	-0.48	1.35
	2007	16.04	2.86	27.01	9.46	87.51	9.89	91006.29	60058.75	41.16	26.10	0.46	0.60
	2008	17.58	4.03	29.43	6.74	90.11	7.70	36168.50	10034.37	41.40	34.65	0.03	0.92
	2009	15.99	3.58	26.00	9.62	88.57	7.80	93680.57	124877.84	56.63	61.04	0.00	1.24
	2010	16.93	3.63	23.34	9.93	91.22	9.04	264311.59	344147.70	56.14	52.75	-0.97	0.56
	2011	17.30	4.14	22.62	10.51	88.75	8.16	102268.78	159326.66	44.58	56.24	-0.42	1.53
	2012	17.87	3.97	27.91	6.88	91.30	6.15	49289.17	56669.82	27.63	24.80	-0.50	1.15

Taxonomic diversity

Species richness showed no significant tendency across time (evaluated with linear regression), but increased with the increase of temperature and salinity and also with the decrease of river runoff (Table 2.4). Species richness also varied significantly between sites, with site M showing highest values. The interaction season x year was also significant in all years, with 2005, 2006 and 2013 showing differences between seasons. In summer, autumn and spring, pairwise differences between years were more pronounced than in winter (Table 7 in Appendix 2).

Table 2.4 - Linear regressions between taxonomic, functional and phylogenetic indices and environmental variables (R^2). ns- non significant values.

Index		Temperature	Salinity	Oxygen	River runoff	Precipitation	NAO Index
Taxonomic	Species richness	0.012	0.022	ns	0.008	ns	ns
	Shannon-Winer	ns	0.012	ns	ns	ns	ns
	Simpson	ns	ns	ns	ns	ns	ns
	Pielou's evenness	0.047	ns	0.011	0.011	ns	ns
Functional	Functionally unique species	0.012	0.022	ns	0.008	ns	ns
	Functional richness	ns	0.115	ns	ns	ns	ns
	Functional evenness	0.012	ns	0.009	ns	ns	ns
	Functional divergence	ns	0.032	0.012	ns	ns	ns
	Functional dispersion	ns	0.150	ns	ns	ns	ns
	Rao's Quadratic Entropy	ns	0.129	ns	ns	ns	ns
Phylogenetic	Rao's Quadratic Entropy	ns	ns	ns	ns	ns	ns
	Mean pairwise distance	0.025	ns	0.008	0.011	ns	ns

Shannon-Wiener diversity index showed a significant relationship with salinity (Table 4) and varied significantly with site, years and the interaction site x season (Table 8 in Appendix 2). Site M and years 2005 and 2013 had the highest values and 2006 the lowest. The interaction site x season highlighted winter as the season with highest pairwise differences among sites, and sites M, S1 and N2 with highest variations among seasons.

Simpson diversity index showed no relevant linear relationship with environmental variables (Table 4), despite its variation between years, where 2005 and 2006 had the highest and lowest values, and a significant site x year interaction, except for differences between sites in 2007 and 2008 (Table 9 in Appendix 2).

Pielou's evenness index showed significant linear relationships with environmental variables, namely a negative relationship with temperature and positive with dissolved oxygen and river runoff – see Table 4. Pielou index varied among sites, seasons and years - site S1 with higher values, summer with lower values when compared to other seasons and years 2005 and 2011 differing the most from other years for their high and low values, respectively (Table 10 in Appendix 2). The significant site x year interaction was mostly due to all years (except 2012) showing differences among sites, and to strong differences among years in all sites, though less in site N2 (Table 10 in Appendix 2).

Functional Diversity

In each of the samples of the studied fish assemblage, all species had different combinations of functional traits, i.e. every species was functionally different from all others – FSing. Therefore,

relationships with environmental variables and variations between sites and significant season x year interaction were the same as shown by species richness (Table 11 in Appendix 2).

Functional richness (FRic) was the only functional diversity index analysed that showed a significant variation through the sampled years, namely a decrease in the functional space occupied by the traits present in the community (R^2=0.01446). In addition, FRic increased with salinity – see Table 4. FRic varied significantly among sites and years - with site M clearly showing highest values and year 2005 showing highest values (Table 12 in Appendix 2). In addition, we found three significant interactions: 1) site x year - where apart from site S2, all sites were different across years, particularly 2003, 2004, 2008, 2012 and 2013, and sites differed in all years except 2006; 2) season x year - with summer, autumn and spring showing more differences between years than winter (more specifically in years 2003, 2005, 2007 and 2013) while all years but 2004, 2005, 2006 and 2008 had significant differences between seasons; 3) site x season x year – see Table 12 in Appendix 2).

Among all functional diversity indices analysed, only functional evenness (FEve) showed no significant variations with salinity (Table 4) even though it showed an inverse relationship with temperature and a direct relationship with dissolved oxygen (Table 13 in Appendix 2). Moreover, functional divergence (FDiv) showed an inverse relationship with salinity and dissolved oxygen (Table 14 in Appendix 2). Both these indices (FEve and FDiv) showed significant variations between sites, with site S1 differing the most from the others in terms of FEve, with highest values (M and N2 showed low mean values), and M the most differing site in terms of FDiv (with lowest value). In addition, FEve differed significantly between years, with 2003 and 2011 standing out for their low values and 2005 for its high values (Table 13 in Appendix 2). Finally, FDiv showed a significant site x season interaction, with summer, autumn and spring showing differences between sites, and only sites S1 and S2 showing differences among seasons (Table 14 in Appendix 2).

Functional dispersion (FDis) and FRaoQ, both of which measure dispersion, showed similar links with environmental variables (Table 4) namely a significant relationship with salinity. Both indices (FDis and FRaoQ) varied among sites, with sites M and S1 (with high values) and N2 (low values) the most different sites, and had two significant interactions: 1) site x season, with summer, autumn and winter again the seasons with more pairwise differences between sites, and sites S1 and N1 the sites with more pairwise differences between seasons, especially autumn and spring, 2) and site x year , with strong differences between years in all sites, except S2 that showed no significant results, and with sites differing in all years, though less markedly in 2006 and 2010 (Tables 15 and 16 in Appendix 2). However, only FDis varied among years – with 2010 the most different year with high values – and had significant season x year interaction, with summer, autumn and spring being the seasons with more differences across years, and the years 2004, 2005, 2009 and 2012 with more differences among seasons (Table 15 in Appendix 2).

Phylogenetic diversity

PRaoQ showed no variation with environmental factors, while mean pairwise distance showed a positive relationship with temperature and negative with oxygen and river runoff (Table 4). Both phylogenetic diversity indices (PRaoQ and mean pairwise distance) varied between years – high values in 2005 and low mean distance in 2013, and had two significant interactions: 1) site x season, with only winter varying among sites and neither S2 and N2 sites differed among seasons in PRaoQ and all seasons differing between sites and site N2 showing no significant variations among seasons in mean pairwise distance; 2) site x year, with all sampling sites differing between years; and in the opposite test, 2007, 2008 and 2009 for PRaoQ and 2004, 2005, 2007 and 2009 for mean pairwise distance showing no

variations among sites (Tables 17 and 18 in Appendix 2). Moreover, mean pairwise distance also varied among sites – low in site S1, and high in S2 and N2 (Table 18 in Appendix 2).

Comparison among taxonomic, functional and phylogenetic diversity

All taxonomic diversity indices were positively correlated, except for species richness and Pielou's evenness (R=0.14). Shannon-Wiener and Simpson index were the most correlated indices (R=0.96) (Table 2.5). Besides, strong positive correlations were also observed for Shannon-Wiener and number of species (R=0.74) and for Simpson index and Pielou's index (R=0.75) (Table 2.5).

Table 2.5 – Pearson correlation (R) among all taxonomic diversity indices measured to characterize the fish assemblages in the Mondego estuary.

Index	Species richness	Simpson	Shannon-Wiener	Pielou's evenness
Species richness	1			
Simpson	0.567	1		
Shannon-Wiener	0.736	0.959	1	
Pielou's evenness	-0.136	0.751	0.549	1

Among functional diversity indices, correlations between FSing and FRic (R=0.78) and between FDis and FRaoQ (R=0.95) were the highest (Table 2.6). Furthermore, correlations between FRic and FRaoQ (R=0.51), between FDis and FSing (R=0.43) and FDis and FRic (R=0.50) were also significant. In addition, negative correlations were found, namely between FSing and FEve (R= -0.28), between FRic and FEve (R= -0.19) and between FDiv and FRic (R= -0.12).

Table 2.6 – Pearson correlation strength (R) for all functional diversity indices measured to characterize the fish assemblages in the Mondego estuary. ns - correlation was not significant.

Index	FSing	FRic	FEve	FDiv	FDis	FRaoQ
FSing	1					
FRic	0.775	1				
FEve	-0.276	-0.188	1			
FDiv	ns	-0.123	ns	1		
FDis	0.429	0.492	0.281	ns	1	
FRaoQ	0.38	0.509	0.247	ns	0.953	1

Phylogenetic diversity indices (RaoQ and Mean pairwise distance) were weakly correlated (R=0.31).

Correlations between the three RaoQ (TRaoQ = Simpson index, FRaoQ and PRaoQ) were significant for all pairwise comparisons, with correlation between PRaoQ and Simpson index notably high (Table 2.7).

Table 2.7 – Pearson correlation strength (R) for the three RaoQ indices (TRaoQ – Simpson index, FRaoQ and PRaoQ), representing each one of the three facets of biodiversity of the fish assemblages in the Mondego estuary.

Index	Simpson	FRaoQ	PRaoQ
Simpson	1		
FRaoQ	0.542	1	
PRaoQ	0.963	0.545	1

Discussion

Most studies about biodiversity focus mainly on taxonomic diversity approaches (e.g., Grubb 1977; Barraclough *et al.* 1995) and more recently with the high technology development also in phylogenetic approaches (e.g., Forest *et al.* 2007; Kellar *et al.* 2015). Moreover, there are several studies focusing on functional diversity approaches (e.g., Mason *et al.* 2007; Mouillot *et al.* 2013), with some studies making direct comparisons between two (e.g., Villéger *et al.* 2010) and even three diversity metrics (e.g., Devictor *et al.* 2010; Meynard *et al.* 2011). Here, we tried to fill the knowledge gap on relationships between biodiversity dimensions by assessing spatial and temporal variability of three dimensions of biodiversity of a fish assemblage, and by identifying links with environmental variables. In this study we used a large time series that resulted from a long term ecological study in the Mondego estuary, an ecosystem of interest that contains fish species with high economical value (Vasconcelos *et al.* 2009; Baptista *et al.* 2013).

In the Mondego estuary, throughout the last decade (2003-2013) salinity increased and dissolved oxygen and North Atlantic Oscilation (NAO) index decreased. Among the analysed diversity indices, only functional richness (FRic) showed a significant slight decline through time, even though this index tended to increase with salinity which increased with time. Salinity acts like an environmental filter (Meynard *et al.* 2011) only allowing the species that are adapted to that salinity range to exist in a given site of the estuary. Despite this loss in functional space (FRic), other characteristics of the community remained with no significant changes, i.e. the fish assemblage kept its taxonomic diversity, the distribution of the abundances of species through the existing functional traits was also unaltered (Functional Evenness and Divergence – Feve and FDiv), the functional differences between species (functionally singular species – Fsing), functional distance between species (Functional Dispersion and Functional RaoQ - FDis and FRaoQ) and its phylogenetic diversity. Nyitrai *et al.* (2012) reported a loss of species richness in the end of their study period (2010), although our results cover a wider time period and suggest no overall negative tendency in any taxonomic diversity measures, which may be related to the incoming of marine species to this estuary, due to an increase in salinity. The previous authors also reported no overall tendencies in evenness, which are corroborated by our results. The decrease in FRic, despite no decrease in none of the other diversity indices and despite the increase in salinity suggests that even though the increase in salinity may allow the fish assemblages to maintain most diversity metrics (including species richness), the set of functional traits present in the community is becoming reduced. In the Mondego estuary, in the last 30 years, almost 20 species have disappeared, most of them freshwater species, due to changes in habitat that increased salinity across the estuary [caused by anthropogenic interventions (Leitão *et al.* 2007)]. One possible explanation for the maintenance of several diversity indices and the decrease in FRic is the disappearance of freshwater species reported by Leitão *et al.* (2007) and their possible replacement by other species taxonomically and phylogenetically different from the existing species in the estuary (i.e. maintaining taxonomic and phylogenetic diversity) but with some functional traits similar to the communities' traits (i.e. decreasing the number of functional traits and the functional space occupied by the community - FRic). Although this was not

directly tested, this could have resulted in the homogenization of the fish assemblages. Homogenization in marine fish assemblages has already been reported for example by Magurran *et al.* (2015), namely the homogenization of marine groundfish assemblages in the north and west seas of Scotland caused by anthropogenic pressures, and Villéger *et al.* (2010) that reported a loss in functional diversity in tropical fish assemblages of an estuary in the Gulf of Mexico, due to a loss of functional specialization, while species richness increased.

In the Mondego fish assemblage, the RaoQ indices [which are dispersion indices, measuring pairwise differences between species (Botta-Dukát 2005; Meynard *et al.* 2011)] for three dimensions of diversity (FRaoQ, PRaoQ and Simpson index) were correlated to each other. Accordingly, several previous studies showed similarity between these three dimensions of diversity. Baptista *et al.* (2015) reported related patterns of taxonomic and functional diversity (species richness and functional richness) in the Mondego estuary, while several studies (e.g., Barker 2002; Rodrigues & Gaston 2002; Schipper *et al.* 2008; Devictor *et al.* 2010) enhanced species richness as an indicator of phylogenetic diversity and other works (e.g., Webb *et al.* 2002; Forest *et al.* 2007; Cadotte *et al.* 2009; Flynn *et al.* 2011; Meynard *et al.* 2011) reported direct relationship between functional and phylogenetic diversities. In addition, these RaoQ indices revealed no overall tendency through time indicating some degree of stability in the Mondego estuary fish assemblage over the study time scale despite inter-annual environmental fluctuations, and in spite of the decrease in FRic described above. It has been previously proposed that phylogenetic diversity is highly linked to functional diversity (Forest *et al.* 2007; Meynard *et al.* 2011) and that higher levels of phylogenetic diversity may allow a community to keep its functional diversity, generating new evolutionary solutions to resist despite changes in the environment (Forest *et al.* 2007; Cadotte *et al.* 2009; Chai *et al.* 2016). Moreover, Forest *et al.* (2007) argued that incorporating gains in phylogenetic diversity is the best strategy to be adopted by conservation plans, since it allows the communities to resist despite changes in the environment in an uncertain future.

In the present study, in all samples species richness was the same as FSing because all fishes were functionally different from each other. We used five functional traits in our analysis (salinity preference, mobility, diet, feeding mode, maximum body length), but if we had used more traits, this relationship between species richness and FSing could have been different and possibly less obvious. However, we chose not to use more functional traits since additional functional traits characterized in a preliminary stage showed high correlations. The number of traits used can influence the results of diversity indices (Petchey & Gaston 2002), therefore traits should be selected with caution.

Although we didn't observe overall tendencies in the measured diversity metrics (except for FRic), inter-annual and seasonal variations were also investigated as well as the relevance of environmental effects in these variations. In this study, the analysed diversity indices responded differently to environmental variables tested. Several indices (species richness, Shannon-Wiener, FSing, FRic, FDis and FRaoQ) were positively affected by the increase in salinity, possibly due to entry to sites and seasons with higher saline influence. Meanwhile FDiv and Pielou were negatively affected. Lower FDiv and Pielou evenness indices mean that species abundance in the communities are not well distributed, with some species representing the majority of the individuals while other species have very few individuals. In this fish assemblage high values of these indices seem to be associated with low temperature and high dissolved oxygen. One possible explanation is the seasonal change in abundance of some of the most abundant species (like the estuarine residents *Pomatoschistus microps* and *Pomatoschistus minutu*s) for which the peak of juveniles is between April and October and between April and November respectively (Nyitrai *et al.* 2013), with lowered abundances of this species in cold season. Although higher temperatures increased species richness, FSing and mean pairwise distance, decreased the regularity in the distribution of species abundances (evenness). Temperature has been increasing over the past years,

increasing the number of warm-water species among the Portuguese coast (Costa *et al.* 2014). In addition, higher temperatures and low river flow and precipitation (are associated with high salinity) have been linked with the increase of species with high affinity with the marine ecosystem in this estuary such as *Arnoglossus laterna*, *Buglossidium luteum and Pegusa lascaris* (Martinho *et al.* 2010; Baptista *et al.* 2015) which may decrease evenness indices since they are not abundant.

In the past few years in the Mondego estuary, several extreme environmental episodes have been reported, namely events of extreme droughts or intense rainy periods, that seriously influence river runoff and by consequence the salinity inside the estuary (Marques *et al.* 2007; Martinho *et al.* 2007b). The year of 2005 (together with 2010) was the warmest of the century (Baptista *et al.* 2015) and had an extreme drought event and decrease in river runoff in the Mondego estuary (Martinho *et al.* 2007b). It is not surprising that several indices showed significant difference between years. The year of 2005 significantly stands out with higher values in all indices with significant differences between years (*i.e.*, Shannon-Wiener, Simpson, Pielou, FRic, FEve, FDis – less clearly, PRaoQ, mean pairwise distance), despite its high temperature and both Pielou and FEve showed negative relationships with temperature. In fact, river runoff affected negatively species richness, FSing and mean pairwise distance while it positively affected Pielou's evenness. Decrease in river runoff seems to result in the increase in the number of marine species in estuaries (due to increase in salinity) (Whitfield & Harrison 2003; Martinho *et al.* 2007b). Elsewhere Livingston (1997) reported the increase in the number of herbivore fishes due to higher light penetration and increase in water column productivity. However, in temporarily open estuaries for example Vivier *et al.* (2010) showed lower species richness in drought years (characterized by low river runoff) since there was no communication between the estuary and the sea, resulting in the decrease in the colonization by marine species and in species richness due to hyperhaline conditions (Simier *et al.* 2004; Peralta-Meixueiro & Vega-Cendejas 2011).

Furthermore, low Shannon-Wiener and Simpson in year 2006 can be related to higher river runoff and precipitation (Nyitrai *et al.* 2012) since river runoff decreases species richness (an index from which Shannon-Wiener and Simpson indices depend on).

Previous studies have shown the effect of environmental factors on estuarine fish assemblages due to seasonal and spatial fluctuations (e.g. longitudinal salinity gradient) (Blaber & Blaber 1980; Rakocinski *et al.* 1992; Marshall & Elliott 1998; Gelwick *et al.* 2001; Akin *et al.* 2003). However, in our study most of the diversity indices didn´t vary significantly between seasons with the exception of Pielou's evenness index - which was lower in summer when temperature was higher and river runoff lower. Reproductive biology of fishes, with the associated recruitment patterns and spawning migrations can possibly help to explain such seasonal variations (Subrahmanyam & Coultas 1980; Potter *et al.* 1986; Drake & Arias 1991; Yoklavich *et al.* 1991; Potter *et al.* 2001; Thiel & Potter 2001; Akin *et al.* 2003), as they lead to changes in fishes abundances, and could contribute to the seasonal variations observed for Pielou index through changes in species abundances. Indeed, most of the fish species that use Portuguese estuaries as nursery areas spawn in sea during winter-spring and their juveniles reach estuaries during spring-summer (Vasconcelos *et al.* 2010) where they attain high densities – this is the case of *Dicentrarchus labrax, Platichthys flesus and Solea solea* in the Mondego estuary (Martinho *et al.* 2007a). Pielou's evenness index was the only analysed diversity index to vary seasonally, suggesting that despite changes in presence and abundance of some species within this fish assemblage over time (Martinho *et al.* 2010) the taxonomic, functional and phylogenetic biodiversity of this community is not marked. This seasonal stability in this estuary is inconsistent with previous studies, namely in freshwater (Tremain & Adams 1995), estuarine (Maes *et al.* 1998; Shimadzu *et al.* 2013) and marine areas (Lazzari *et al.* 1999), where high seasonal variations were reported.

In addition to temporal variations, diversity indices were sensitive to spatial differences within the Mondego estuary. The five sampled areas of the Mondego estuary represent a longitudinal salinity gradient (lower salinity in sites N2 and S2, and higher values for sites M and S1) and they comprise different habitats and depths. These are well known structuring environmental factors for fish assemblages within estuaries (species richness and abundance) - especially salinity (Marshall & Elliott 1998; Leitão *et al.* 2007; Elliott & Whitfield 2011; França *et al.* 2011), which is corroborated by our results, since salinity showed stronger correlations with many diversity indices that other environmental effects, although still with weak correlations. Site M (closer to the ocean and highest salinity) had higher values of several diversity indices: high species richness and Shannon-Wiener, and also bigger functional space filled by the communities (FRic) and numerous functionally different species (FSing, FDis and FRaoQ). Moreover, site N2, with lowest salinity, showed overall low values for those same indices. These results suggest a spatial gradient of high functional and taxonomic diversity from the mouth to lower diversity upstream within Mondego estuary. Leitão *et al.* (2007) reported that in this estuary, lower areas were associated to marine and nursery species, while catadromous and freshwater species were mainly present in the upper estuary where salinity is lower, which could explain this spatial gradient in the Mondego estuary, since the majority of the fish species present are marine (Nyitrai *et al.* 2012; Baptista *et al.* 2015). Therefore, in this case, higher functional diversity is possible related to higher taxonomic diversity that exists in site M, since estuarine areas have lower diversity than other aquatic systems (Elliott & Whitfield 2011). In fact, species richness and FRic followed similar patterns through all sampling sites, as reported by Baptista *et al.* (2015). However, site M located downstream has low values of both FEve and Pielou's evenness (but higher taxonomic and functional richness) possibly suggesting that there is a small number of species and traits with the majority of the community abundances. In contrast, phylogenetic diversity showed a different pattern since only mean pairwise distance differed between sites, suggesting that phylogenetic differences between species are very low in site S1, while higher phylogenetic differences between species occur in sites S2 and N2 where salinity is lower. However, site M has higher phylogenetic diversity than site S1. This suggests that in the case of phylogenetic diversity a spatial gradient is not clear and our results suggest that higher phylogenetic diversity is found in the lower and upstream areas of the estuary (sites M, S2 and N2 respectively), while in the transition zone between lower and upstream areas, low phylogenetic diversity is found. This may be explained by the already mentioned higher diversity in the marine and freshwater areas than inside the estuary (Elliott & Whitfield 2011). As some previous authors have showed, high phylogenetic diversity should mean high functional diversity too (Forest *et al.* 2007; Cadotte *et al.* 2009; Devictor *et al.* 2010).

Our results revealed new perspectives on the variation of diversity indices, and further strengthens knowledge about estuarine fish communities. However, it is likely that other factors affect the fish assemblages in the Mondego estuary, such as anthropogenic pressures, as reported by several authors (e.g., Marques *et al.* 1993; Castro & Freitas 2006; Dolbeth *et al.* 2007; Vasconcelos *et al.* 2007), and which were not accounted within this study and might help to improve the explanations about the patterns observed for diversity indices. In the future, more studies about estuarine fish assemblages should be developed to monitor biodiversity, and considering the high economic importance of their species (Vasconcelos *et al.* 2009) and the ecosystem services they provide to humans (Díaz *et al.* 2007), management and conservations actions should be implemented to avoid severe biodiversity losses.

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CHAPTER 3

Final remarks

Final remarks

Biodiversity loss is a worldwide concern (Loh *et al.* 2005; Worm *et al.* 2006; Dornelas *et al.* 2014) including in ecosystems such as estuaries (Lotze *et al.* 2006; Worm *et al.* 2006). In the Mondego estuary, the fish assemblage has gone through changes in species composition in the last decades, mainly due to the increase in salinity, resulting in severe decline in the number of freshwater species (Leitão *et al.* 2007; Nyitrai *et al.* 2012). But more recently, throughout the study period (2003-2013) the fish assemblage of the Mondego estuary has mostly kept its overall diversity despite inter-annual and seasonal fluctuations. The increase in salinity in this estuary (Leitão *et al.* 2007), results in an increased colonization by marine species (Martinho *et al.* 2010; Baptista *et al.* 2015), which can counteract the systematic diversity loss of freshwater species in upstream area. Similarly, Dornelas *et al.* (2014) showed that changes in assemblage composition (with the disappearance of some species and appearance of others) can result in no net diversity loss. However, they measured it only taxonomically which can mask real changes that can be happening functionally (Villéger *et al.* 2010).

Moreover, our study shows that in addition to species richness, functional and phylogenetic diversity also didn't show a linear trend throughout this study period (despite a small loss in functional richness). This decrease in the functional richness, was probably related to the already reported decrease in the number of freshwater species (Leitão *et al.* 2007; Nyitrai *et al.* 2012). Increase in salinity increases the number of marine species inside the estuary, which may result in the homogenization of the fish assemblage, as reported by Magurran *et al.* (2015) for marine assemblages due to climate change. Despite the decrease in functional richness, our results enhance the stability of the fish assemblage in the Mondego estuary. Other studies have showed stability of some facets of diversity (taxonomic) despite changes in other facets (functional) (e.g., Villéger *et al.* 2010).

Furthermore, phylogenetic diversity has been linked by some authors to the other diversity facets (Barker 2002; Cadotte *et al.* 2009; Devictor *et al.* 2010; Flynn *et al.* 2011), and some have shown that maintaining phylogenetic diversity may allow communities to keep taxonomic and functional diversity (Forest *et al.* 2007; Meynard *et al.* 2011), and in our study there were no changes in phylogenetic diversity. Moreover, the natural tolerance to environmental changes that characterize estuarine organisms (Elliott & Whitfield 2011) could render them more resilient to environmental and anthropogenic pressures.

Regarding seasonal variations, in the Mondego estuary, only taxonomic evenness (measured with Pielou index) showed seasonality, possible due to annual life cycles of abundant species (Subrahmanyam & Coultas 1980; Potter *et al.* 1986; Drake & Arias 1991; Yoklavich *et al.* 1991; Potter *et al.* 2001; Thiel & Potter 2001; Vasconcelos *et al.* 2010). This results contrasts with other systems and studies where seasonal variations have been reported (e.g., Marshall & Elliott 1998; Akin *et al.* 2003; Shimadzu *et al.* 2013), namely in terms of species dominance which may also contribute to maintain biodiversity including through seasonal alternated dominance of species (e.g., Shimadzu *et al.* 2013).

Regarding environmental effects, the results of this study are supported by previous studies, where salinity, temperature and river runoff were the factors that most influenced the fish assemblages in estuaries (e.g., Livingston 1997; Martinho *et al.* 2007; Elliott & Whitfield 2011; França *et al.* 2011).

Furthermore, the spatial gradients (in terms of richness) also reported by the bibliography (e.g., Marshall & Elliott 1998; Leitão *et al.* 2007) were also verified for both taxonomic and functional diversities. In contrast, phylogenetic diversity exhibited a different pattern, since it was higher in the mouth and upstream areas and lower in the middle estuary, which is probably related to higher diversity in freshwater and marine ecosystems adjacent to estuaries (Elliott & Whitfield 2011).

Inverse spatial patterns of richness and evenness are very notorious in our results, implying that where diversity is higher species abundances aren't well distributed (sites M and N2 - lower and upstream estuary respectively), reflecting dominance by some species regarding others. In the Mondego estuary the most dominant species are estuarine residents *Pomatoshchistus microps* and *Pomatoschistus minutus,* and four marine migrant species: *Solea solea, Dicentrarchus labrax, Platichthys flesus* (Nyitrai *et al.* 2012) that use this estuary as a nursery ground in high abundances, and also of *D. vulgaris which has* increased its abundance in the Mondego estuary due the increase in salinity.

Moreover, our study also shows strong relationships between the three facets of diversity (FRaoQ, PRaoQ and Simpson index) in agreement with the literature (e.g., Forest *et al.* 2007; Cadotte *et al.* 2009; Devictor *et al.* 2010; Meynard *et al.* 2011; Baptista *et al.* 2015), in particular previously reported for species and functional richness of this same fish assemblage using a larger dataset (Baptista *et al.* 2015). In our analysis, these three indices were significantly correlated to each other, especially Simpson and PRaoQ, which is in agreement with previous studies that reported high relationships between taxonomic and phylogenetic diversities (e.g., Barker 2002; Rodrigues & Gaston 2002; Schipper *et al.* 2008).

Our study brings new perspectives to the changes and drivers of biodiversity of the fish assemblage in this estuary. However, we didn't take into account changes in anthropogenic pressures that can also be responsible for some of the patterns observed (Dolbeth *et al.* 2007). The Mondego estuary has been documented to have considerable anthropogenic pressures (Neto *et al.* 2010; Verdelhos *et al.* 2014), even though in Portugal many other estuaries showed higher anthropogenic impacts (Vasconcelos *et al.* 2007). In addition, we used a small set of traits (five) with little overlap and aiming at the association of an ecological significance to each trait used. But a characterization of a different set of functional traits could further improve a characterization of this assemblage, since the number and choice of traits can influence the results (Petchey & Gaston 2002)

In the future, more studies regarding fish assemblages are needed to monitor diversity. Moreover, knowledge of changes in functional traits (that underlie the diversity indices) is needed. Such studies are essential to identify the need for management interventions in case of severe loss.

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Appendix 1

Table 1 – Definitions regarding each functional trait and subsequent categories and its description

Trait	Definition	Categories	Description
Salinity preference	Reflects the ability to deal with physiological osmotic stress regarding the salinity changing nature of transitional waters	Marine	Species that prefer salinities higher than 30
		Brackish	Species that tolerate a wide range of salinities (5-30)
		Euryhaline	Species migrating between fresh and salt water in one or more life stage, tolerating a wide range of salinities
		Freshwater	Species that prefer salinities between 0 and 0.5
Mobility	The ability of fish to move, which reflects the capacity of fish to respond to local changes in habitat	High	Movement over large distances
		Medium	Daily movement of tens of meters
		Sedentary	Limited movement and well defined home ranges
		Territorial	Limited movement and territorial behavior
Diet	Defined based on the fish diet, it reflects trophic structure, distribution of resources and how organisms may adapt to the habitat	Invertebrate feeders	Eat invertebrates such as mollusks and small crustaceans - includes zoobenthivores

Table 1 (continued) - Definitions regarding each functional trait and subsequent categories and its description.

Trait	Definition	Categories	Description
Feeding mode	Primary method of feeding used by the species	Omnivorous	Feeding predominantly on filamentous algae, macrophytes, periphyton, epifauna and infauna
		Detritivorous	Feeding predominantly on detritus and/or microphytobenthos
		Planktivorous	Feeding predominantly on plankton (e.g. Hydroids, planktonic crustaceans, fish eggs/larvae)
		Herbivorous	Grazing predominantly on living macroalgal and macrophyte material or phytoplankton
		Macrocarnivorous	Feeding on macroinvertebrates and vertebrates (mostly fish)
		browser	browsing on substrate; selecting plankton feeding
		filterer	filtering plankton; sucking food-containing material
		grazer	grazing on aquatic plants
		hunter	hunting macrofauna (predator)
		parasite	feeding on a host (parasite); picking parasites off a host (cleaner)
Maximum body length	Related to other life-history traits, energy and impacts on the food web	No classes were used in this trait. Maximum body size of each species was used.	

Table 2 – Primers and PCR conditions used to amplify the 16S and COI gene fragments.

	16S	COI
Primers	16SFor: AAGCCTCGCCTGTTTACCAA 16SRev: CTGAACTCAGATCACGTAGG (Almada et al. 2005)	94º4' + 30x (94º 1' + 55º1' + 72º 1') + 72º10'
PCR conditions	COI-F1: TCA ACC ACC CAC AAA GAC ATT GGC AC COI-R2: ACT TCA GGG TGA CCG AAG AAT CAG AA (Ward et al. 2005)	94º3' + 5x (94º 1' + 60-56º touchdown 1' + 72º 1') + 30x (92º45'' + 55º1' + 72º1') + 72º10'

Appendix 2

Table 1 – Results of PERMANOVA main and pairwise tests for temperature, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Site	31.277	0.000	M, S1		4.143	0.003
			M, S2		7.443	0.000
			M, N1		5.063	0.001
			M, N2		6.489	0.000
			S1, S2		5.808	0.001
			S1, N2		3.203	0.012
			S2, N1		5.440	0.001
			S2, N2		4.386	0.003
			N1, N2		4.024	0.004
Season	46.435	0.000	Summer, Autumn		3.855	0.009
			Summer, Winter		13.310	0.000
			Summer, Spring		4.873	0.001
			Autumn, Winter		6.561	0.001
			Winter, Spring		5.995	0.001
Year	3.308	0.001	2003, 2005		3.916	0.000
			2003, 2009		2.169	0.040
			2004, 2010		2.086	0.049
			2005, 2006		3.982	0.000
			2005, 2010		3.097	0.003
			2005, 2012		4.239	0.000
			2006, 2009		2.871	0.008
			2007, 2012		2.079	0.047
			2009, 2010		2.450	0.025
			2009, 2012		3.332	0.004
Site x Season	11.391	0.000	Summer	M, S2	10.929	0.000
				M, N1	6.603	0.001
				M, N2	11.346	0.000
				S1, S2	7.007	0.000
				S1, N2	4.689	0.002
				S2, N1	7.408	0.000
				S2, N2	2.830	0.024
				N1, N2	6.281	0.000
			Autumn	M, S2	2.640	0.036
			Winter	M, S1	2.466	0.045

Table 1 (continued) – Results of PERMANOVA main and pairwise tests for temperature, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				M, N2	4.555	0.005
				S1, N1	4.118	0.004
				S1, N2	9.290	0.000
				S2, N2	2.684	0.016
				N1, N2	2.919	0.023
			Spring	M, S2	7.380	0.000
				M, N1	3.764	0.008
				M, N2	5.569	0.001
				S1, S2	6.044	0.001
				S1, N2	4.466	0.003
				S2, N1	5.996	0.001
				S2, N2	3.683	0.006
				N1, N2	4.299	0.004
			M	Summer, Winter	6.970	0.000
				Summer, Spring	4.286	0.002
				Autumn, Winter	5.943	0.002
				Winter, Spring	4.103	0.008
			S1	Summer, Winter	7.299	0.000
				Summer, Spring	2.557	0.022
				Autumn, Winter	6.517	0.000
				Winter, Spring	3.658	0.009
			S2	Summer, Autumn	6.016	0.001
				Summer, Winter	10.958	0.000
				Summer, Spring	3.562	0.007
				Autumn, Winter	3.355	0.015
				Winter, Spring	4.395	0.003
			N1	Summer, Winter	12.762	0.000
				Summer, Spring	3.982	0.007
				Autumn, Winter	7.638	0.001
				Winter, Spring	6.472	0.001
			N2	Summer, Autumn	4.617	0.004
				Summer, Winter	13.741	0.000
				Summer, Spring	5.269	0.001
				Autumn, Winter	8.289	0.000
				Winter, Spring	7.578	0.000
Season x Year	2.490	0.000	2003	Summer, Autumn	4.434	0.001
				Summer, Winter	11.912	0.000
				Autumn, Winter	3.471	0.005
			2004	Summer, Winter	12.295	0.000
				Winter, Spring	6.111	0.000
			2005	Summer, Autumn	5.327	0.000
				Summer, Winter	11.974	0.000

Table 1 (continued) – Results of PERMANOVA main and pairwise tests for temperature, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				Summer, Spring	3.341	0.004
				Autumn, Winter	4.368	0.001
				Autumn, Spring	2.686	0.017
				Winter, Spring	14.864	0.000
			2006	Summer, Winter	9.326	0.000
				Autumn, Winter	4.835	0.001
				Winter, Spring	11.992	0.000
			2008	Summer, Winter	4.823	0.006
				Summer, Spring	4.131	0.009
			2009	Autumn, Winter	5.215	0.001
				Winter, Spring	4.066	0.009
			2010	Summer, Winter	11.155	0.010
				Summer, Spring	7.516	0.003
				Autumn, Winter	4.997	0.021
				Autumn, Spring	5.673	0.009
				Winter, Spring	14.365	0.000
			2011	Summer, Winter	10.733	0.002
				Winter, Spring	3.548	0.033
			2012	Summer, Autumn	4.660	0.006
				Summer, Winter	26.683	0.003
				Summer, Spring	3.068	0.015
				Autumn, Spring	2.805	0.038
				Winter, Spring	3.879	0.010
			Summer	2003, 2004	2.907	0.014
				2003, 2007	3.348	0.004
				2003, 2009	2.761	0.015
				2003, 2011	3.652	0.003
				2003, 2012	3.402	0.004
				2007, 2012	3.232	0.022
				2010, 2012	2.931	0.031
			Autumn	2003, 2005	2.480	0.024
				2003, 2012	2.143	0.043
				2005, 2006	2.763	0.015
				2005, 2012	3.569	0.004
				2007, 2012	3.540	0.005
				2009, 2012	3.267	0.006
			Winter	2003, 2005	4.245	0.009
				2004, 2005	5.428	0.000
				2004, 2007	6.396	0.000
				2004, 2008	4.093	0.001
				2004, 2009	2.968	0.009
				2005, 2006	3.666	0.008

Table 1 (continued) – Results of PERMANOVA main and pairwise tests for temperature, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2005, 2007	9.083	0.003
				2005, 2008	7.161	0.003
				2005, 2010	5.719	0.001
				2005, 2011	3.779	0.010
				2005, 2012	5.014	0.006
				2006, 2007	10.426	0.004
				2006, 2008	5.893	0.010
				2006, 2009	2.690	0.049
				2006, 2010	3.006	0.022
				2006, 2012	2.811	0.043
				2007, 2010	3.479	0.048
				2009, 2010	3.536	0.043
			Spring	2004, 2006	2.437	0.028
				2005, 2006	3.944	0.002
				2005, 2007	2.176	0.046
				2005, 2008	3.475	0.005
				2005, 2010	2.524	0.022
				2006, 2007	4.267	0.002
				2006, 2008	5.445	0.005
				2006, 2009	3.474	0.006
				2006, 2010	2.359	0.044
				2006, 2012	2.636	0.026
				2007, 2010	3.443	0.008
				2008, 2010	13.620	0.002
				2009, 2010	2.538	0.033

Table 2 – Results of PERMANOVA main and pairwise tests for salinity, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Site	93.280	0.000	M, S2		5.952	0.000
			M, N1		6.554	0.000
			M, N2		12.170	0.000
			S1, S2		6.125	0.001
			S1, N1		7.535	0.000
			S1, N2		15.784	0.000
			S2, N2		10.353	0.000
			N1, N2		10.772	0.000
Season	4.754	0.010	Summer, Winter		3.718	0.004
			Summer, Spring		5.856	0.000
Year	8.026	0.000	2003, 2005		4.211	0.000
			2003, 2007		4.847	0.000
			2003, 2008		4.919	0.000
			2003, 2009		2.231	0.032
			2003, 2012		5.138	0.000
			2004, 2005		5.081	0.000
			2004, 2007		2.951	0.006
			2004, 2008		7.047	0.000
			2004, 2012		4.755	0.000
			2005, 2006		2.670	0.010
			2005, 2008		2.032	0.046
			2005, 2010		3.088	0.004
			2005, 2011		3.750	0.001
			2005, 2012		2.552	0.013
			2006, 2007		2.898	0.009
			2006, 2008		2.192	0.039
			2006, 2012		3.125	0.005
			2007, 2008		4.512	0.000
			2007, 2010		4.040	0.001
			2007, 2011		4.874	0.000
			2008, 2010		6.473	0.000
			2008, 2011		6.819	0.000
			2009, 2011		2.288	0.030
			2010, 2012		4.075	0.001
			2011, 2012		4.921	0.000
Season x Year	3.171	0.000	2004	Summer, Winter	2.927	0.011
				Summer, Spring	1.643	0.125
				Winter, Spring	2.515	0.021
			2005	Summer, Autumn	4.224	0.001
				Summer, Spring	2.106	0.048
				Autumn, Winter	2.667	0.022
				Autumn, Spring	2.156	0.047

Table 2 (continued) – Results of PERMANOVA main and pairwise tests for salinity, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
			2006	Summer, Autumn	2.332	0.041
			2007	Summer, Autumn	3.635	0.003
				Autumn, Winter	6.617	0.000
				Autumn, Spring	5.405	0.000
			2008	Summer, Winter	3.338	0.018
			2009	Autumn, Winter	2.383	0.028
			2010	Summer, Winter	9.338	0.011
				Winter, Spring	6.262	0.000
			2011	Summer, Winter	7.177	0.002
				Summer, Spring	3.883	0.005
				Autumn, Winter	4.019	0.007
				Winter, Spring	5.069	0.016
			2012	Summer, Winter	6.842	0.003
			Summer	2003, 2004	2.224	0.044
				2003, 2005	5.898	0.000
				2003, 2006	4.451	0.001
				2003, 2007	3.409	0.004
				2003, 2008	7.644	0.000
				2003, 2009	4.705	0.001
				2003, 2010	3.508	0.003
				2003, 2011	5.197	0.000
				2003, 2012	7.237	0.000
				2004, 2008	2.924	0.016
				2004, 2012	2.608	0.027
				2006, 2008	4.565	0.002
				2006, 2012	4.049	0.003
				2007, 2008	5.091	0.004
				2007, 2012	8.471	0.003
				2008, 2010	4.784	0.006
				2010, 2012	7.820	0.002
			Autumn	2003, 2007	4.769	0.000
				2003, 2009	2.145	0.046
				2003, 2012	2.420	0.031
				2005, 2007	5.940	0.000
				2005, 2009	2.312	0.033
				2005, 2012	3.043	0.011
				2006, 2007	4.125	0.001
				2006, 2009	2.663	0.015
				2007, 2010	9.678	0.000
				2007, 2011	4.939	0.001
			Winter	2003, 2005	3.057	0.025
				2003, 2010	3.149	0.049

Table 2 (continued) – Results of PERMANOVA main and pairwise tests for salinity, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2004, 2005	4.873	0.000
				2004, 2007	2.612	0.020
				2004, 2008	4.693	0.000
				2004, 2011	2.430	0.036
				2004, 2012	4.374	0.001
				2005, 2006	2.337	0.046
				2005, 2009	3.838	0.011
				2005, 2010	6.179	0.000
				2005, 2011	5.350	0.004
				2007, 2010	6.825	0.015
				2008, 2010	10.067	0.008
				2010, 2012	9.570	0.011
			Spring	2004, 2005	2.852	0.009
				2004, 2008	5.081	0.000
				2004, 2009	2.441	0.025
				2004, 2010	3.398	0.004
				2008, 2011	4.085	0.029
				2010, 2011	2.489	0.037

Table 3 – Results of PERMANOVA main and pairwise tests for oxygen, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Site	14.070	0.000	M, S2		3.847	0.006
			M, N1		3.857	0.005
			M, N2		4.605	0.002
			S1, S2		6.313	0.000
			S1, N1		3.976	0.004
			S1, N2		6.014	0.000
			N1, N2		3.576	0.008
Season	11.584	0.000	Summer, Winter		3.679	0.007
			Summer, Spring		3.227	0.011
			Autumn, Winter		9.400	0.000
			Autumn, Spring		4.569	0.006
Year	2.588	0.007	2003, 2007		2.366	0.025
			2003, 2008		2.143	0.047
			2003, 2009		3.483	0.001
			2003, 2011		2.438	0.023
			2005, 2006		2.662	0.010
			2005, 2007		2.803	0.008
			2005, 2008		2.302	0.026
			2005, 2009		3.709	0.000
			2005, 2011		2.295	0.028
			2009, 2012		2.480	0.021
Season x Year	1.730	0.027	2003	Summer, Winter	4.569	0.001
			2005	Summer, Winter	4.057	0.000
				Summer, Spring	2.490	0.023
				Autumn, Winter	2.531	0.025
				Winter, Spring	2.380	0.035
			2006	Summer, Autumn	2.244	0.047
				Autumn, Winter	2.534	0.029
				Autumn, Spring	2.262	0.047
			2007	Autumn, Winter	2.197	0.049
			2009	Summer, Autumn	3.937	0.002
				Summer, Spring	5.934	0.004
			2010	Summer, Winter	10.694	0.012
				Autumn, Winter	5.027	0.022
			2011	Summer, Spring	2.746	0.028
				Autumn, Winter	4.348	0.006
				Autumn, Spring	5.092	0.001
			Summer	2003, 2009	4.076	0.002
				2003, 2010	2.780	0.014
				2005, 2009	3.435	0.002
				2005, 2010	2.437	0.021
				2006, 2009	2.611	0.044

Table 3 (continued) – Results of PERMANOVA main and pairwise tests for oxygen, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2007, 2012	2.973	0.033
				2009, 2011	2.668	0.042
				2009, 2012	5.001	0.006
				2010, 2012	3.852	0.012
			Autumn	2006, 2009	2.208	0.042
				2007, 2009	2.093	0.047
				2009, 2011	2.411	0.029
			Winter	2003, 2004	2.483	0.029
				2004, 2005	3.115	0.006
				2004, 2010	2.716	0.019
				2005, 2008	2.830	0.031
				2007, 2010	3.683	0.040
				2008, 2010	7.129	0.015
				2009, 2010	4.040	0.032
				2010, 2011	3.470	0.038
			Spring	2005, 2006	2.881	0.011
				2005, 2009	2.480	0.024
				2005, 2012	2.714	0.020

Table 4 – Results of PERMANOVA main and pairwise tests for river runoff, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Season	4.705	0.008	Summer, Winter		2.917	0.015
			Summer, Spring		3.310	0.011
Year	14.416	0.000	2003, 2004		4.423	0.000
			2003, 2005		8.332	0.000
			2003, 2007		5.090	0.000
			2003, 2008		6.129	0.000
			2003, 2012		6.327	0.000
			2004, 2005		5.151	0.000
			2004, 2008		3.498	0.001
			2004, 2009		3.303	0.003
			2004, 2010		8.519	0.000
			2004, 2011		5.394	0.000
			2004, 2012		2.887	0.007
			2005, 2006		2.858	0.008
			2005, 2007		4.058	0.001
			2005, 2009		6.819	0.000
			2005, 2010		10.756	0.000
			2005, 2011		9.320	0.000
			2007, 2008		5.886	0.000
			2007, 2009		2.873	0.008
			2007, 2010		6.609	0.000
			2007, 2011		6.556	0.000
			2007, 2012		3.387	0.002
			2008, 2009		11.443	0.000
			2008, 2010		10.445	0.000
			2008, 2011		43.986	0.000
			2009, 2010		5.336	0.000
			2009, 2011		3.808	0.001
			2009, 2012		5.791	0.000
			2010, 2011		4.001	0.001
			2010, 2012		7.430	0.000
			2011, 2012		8.277	0.000
Season x Year	16.989	0.000	2003	Summer, Autumn	2.114	0.050
				Summer, Winter	1845.200	0.000
				Autumn, Winter	4.212	0.002
			2004	Summer, Winter	4.130	0.001
				Winter, Spring	4.487	0.001
			2005	Summer, Autumn	2.659	0.015
			2006	Summer, Autumn	2.203	0.049
				Summer, Winter	7.395	0.000
				Summer, Spring	2.485	0.038
			2007	Summer, Spring	2.149	0.068

Table 4 (continued) – Results of PERMANOVA main and pairwise tests for river runoff, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				Autumn, Winter	8.937	0.000
				Autumn, Spring	5.554	0.000
			2009	Autumn, Winter	48.153	0.000
				Autumn, Spring	3.153	0.009
				Winter, Spring	8.851	0.002
			2010	Summer, Winter	8.017	0.007
				Summer, Spring	2.977	0.031
				Autumn, Winter	7.340	0.009
				Autumn, Spring	5.719	0.003
				Winter, Spring	15.219	0.000
			2011	Summer, Autumn	2.463	0.043
				Summer, Winter	728.310	0.000
				Summer, Spring	3.844	0.013
				Autumn, Winter	71.933	0.000
				Autumn, Spring	4.181	0.004
				Winter, Spring	49.048	0.001
			2012	Summer, Winter	7.712	0.002
			Summer	2003, 2004	3.928	0.003
				2003, 2005	9.370	0.000
				2003, 2006	89.902	0.000
				2003, 2007	65.277	0.000
				2003, 2008	4.378	0.002
				2003, 2009	28.297	0.000
				2003, 2010	14.116	0.000
				2003, 2011	20.197	0.000
				2003, 2012	16.184	0.000
				2004, 2005	5.671	0.000
				2004, 2006	17.521	0.000
				2004, 2007	10.191	0.002
				2004, 2011	2.795	0.024
				2004, 2012	7.360	0.000
				2005, 2007	8.733	0.000
				2005, 2009	3.330	0.004
				2005, 2010	4.148	0.001
				2005, 2011	5.219	0.000
				2006, 2007	88.380	0.000
				2006, 2009	34.796	0.000
				2006, 2010	42.917	0.000
				2006, 2011	36.537	0.000
				2006, 2012	3.983	0.005
				2007, 2008	3.939	0.008
				2007, 2011	36.532	0.000

Table 4 (continued) – Results of PERMANOVA main and pairwise tests for river runoff, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2007, 2012	13.933	0.003
				2009, 2012	4.068	0.009
				2010, 2011	4.460	0.007
				2010, 2012	5.563	0.002
				2011, 2012	7.372	0.000
			Autumn	2003, 2007	2.164	0.045
				2003, 2009	2.626	0.019
				2005, 2009	2.252	0.042
				2006, 2007	2.585	0.022
				2006, 2009	2.895	0.012
			Winter	2003, 2004	5.071	0.001
				2003, 2005	102.110	0.000
				2003, 2006	21.142	0.001
				2003, 2010	3.333	0.040
				2004, 2005	5.046	0.001
				2004, 2006	2.529	0.030
				2004, 2008	3.199	0.005
				2004, 2009	3.288	0.006
				2004, 2010	10.735	0.000
				2004, 2011	5.095	0.001
				2004, 2012	3.469	0.003
				2005, 2006	7.134	0.000
				2005, 2007	25.853	0.000
				2005, 2009	80.264	0.001
				2005, 2010	15.928	0.000
				2005, 2011	98.182	0.000
				2006, 2008	3.875	0.014
				2006, 2009	15.749	0.001
				2006, 2010	11.900	0.000
				2006, 2011	19.948	0.001
				2006, 2012	4.690	0.010
				2007, 2010	6.977	0.009
				2008, 2010	8.176	0.007
				2009, 2010	4.377	0.025
				2010, 2012	8.334	0.006
			Spring	2004, 2005	3.095	0.006
				2004, 2007	3.399	0.005
				2005, 2006	2.964	0.016
				2005, 2007	6.299	0.001
				2005, 2008	29.779	0.000
				2005, 2009	2.890	0.017
				2005, 2010	12.855	0.000

Table 4 (continued) – Results of PERMANOVA main and pairwise tests for river runoff, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2005, 2011	8.629	0.000
				2005, 2012	2.359	0.027
				2007, 2009	2.409	0.036
				2007, 2010	3.835	0.007
				2007, 2011	3.151	0.016

Table 5 – Results of PERMANOVA main and pairwise tests for precipitation, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Season	9.055	0.001	Summer, Autumn		5.382	0.002
			Summer, Winter		3.538	0.007
			Summer, Spring		3.969	0.005
Year	2.718	0.010	2003, 2005		2.244	0.033
			2004, 2005		5.772	0.000
			2004, 2012		3.722	0.001
			2005, 2006		4.978	0.000
			2005, 2008		7.692	0.000
			2005, 2009		2.152	0.040
			2005, 2010		8.790	0.000
			2005, 2012		4.087	0.000
			2006, 2007		3.393	0.002
			2006, 2012		4.390	0.001
			2007, 2010		3.237	0.004
			2008, 2010		2.932	0.010
			2008, 2012		3.081	0.008
			2009, 2012		2.300	0.033
			2010, 2012		4.857	0.000
Season x Year	4.690	0.000	2003	Summer, Autumn	3.553	0.003
				Summer, Winter	5.391	0.001
			2004	Summer, Winter	3.699	0.004
				Winter, Spring	8.687	0.000
			2005	Summer, Autumn	30.428	0.000
				Summer, Winter	3.732	0.002
				Summer, Spring	20.074	0.000
				Autumn, Winter	19.481	0.000
				Autumn, Spring	6.777	0.000
				Winter, Spring	11.979	0.000
			2006	Summer, Autumn	6.297	0.000
				Summer, Winter	3.305	0.012
				Autumn, Winter	3.924	0.004
				Autumn, Spring	5.256	0.001
			2007	Autumn, Spring	2.291	0.041
			2008	Summer, Winter	9.377	0.002
				Summer, Spring	2.030	0.077
			2009	Summer, Spring	11.025	0.003
				Autumn, Winter	2.374	0.028
				Winter, Spring	27.989	0.001
			2010	Summer, Winter	9.502	0.006
				Summer, Spring	8.668	0.002
				Autumn, Winter	4.370	0.023
				Autumn, Spring	50.766	0.001

Table 5 (continued) – Results of PERMANOVA main and pairwise tests for precipitation, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				Winter, Spring	14.108	0.000
			2011	Summer, Autumn	2.357	0.039
				Summer, Winter	48.902	0.000
				Summer, Spring	2.753	0.035
				Winter, Spring	3.703	0.027
			2012	Summer, Autumn	5.156	0.003
				Summer, Spring	2.302	0.045
			Summer	2003, 2005	3.834	0.001
				2003, 2006	4.323	0.001
				2003, 2009	3.214	0.004
				2003, 2010	3.214	0.005
				2003, 2011	3.659	0.003
				2004, 2005	3.639	0.004
				2004, 2011	2.612	0.036
				2005, 2006	12.740	0.000
				2005, 2007	6.978	0.000
				2005, 2008	2.606	0.018
				2005, 2009	2.400	0.027
				2005, 2010	2.400	0.031
				2006, 2007	3.253	0.016
				2006, 2008	3.687	0.007
				2006, 2009	18.435	0.001
				2006, 2010	18.435	0.001
				2006, 2011	21.581	0.000
				2006, 2012	6.452	0.000
				2007, 2011	15.879	0.003
				2007, 2012	2.914	0.037
				2008, 2011	2.460	0.039
				2009, 2011	3.098	0.025
				2010, 2011	3.098	0.024
			Autumn	2003, 2007	2.827	0.011
				2005, 2006	4.239	0.001
				2005, 2007	6.588	0.000
				2005, 2010	9.933	0.000
				2005, 2012	9.110	0.000
				2006, 2007	6.878	0.000
				2006, 2009	3.536	0.004
				2006, 2012	3.611	0.006
				2007, 2010	6.383	0.000
			Winter	2003, 2004	5.860	0.000
				2003, 2005	7.924	0.001
				2003, 2010	4.345	0.024

Table 5 (continued) – Results of PERMANOVA main and pairwise tests for precipitation, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2004, 2005	20.528	0.000
				2004, 2007	22.667	0.000
				2004, 2008	26.769	0.000
				2004, 2009	64.813	0.000
				2004, 2010	8.944	0.000
				2004, 2011	19.226	0.000
				2004, 2012	31.598	0.000
				2005, 2006	5.377	0.001
				2005, 2008	18.915	0.000
				2005, 2009	31.730	0.000
				2005, 2010	12.973	0.000
				2005, 2011	15.329	0.001
				2006, 2009	6.227	0.007
				2006, 2010	3.001	0.023
				2006, 2012	3.339	0.021
				2007, 2010	6.781	0.011
				2009, 2010	5.896	0.014
				2010, 2012	8.075	0.009
			Spring	2004, 2005	6.671	0.000
				2004, 2007	2.606	0.020
				2004, 2010	8.257	0.000
				2005, 2006	2.300	0.040
				2005, 2008	5.537	0.001
				2005, 2009	5.400	0.001
				2005, 2010	12.976	0.000
				2005, 2011	4.531	0.001
				2007, 2010	3.881	0.007
				2008, 2010	7.193	0.002
				2009, 2010	9.326	0.000

Table 6 – Results of PERMANOVA main and pairwise tests for NAO index, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Year	3.599	0.000	2003, 2004		3.056	0.003
			2003, 2006		2.288	0.027
			2003, 2009		3.777	0.000
			2003, 2010		5.460	0.000
			2003, 2011		4.231	0.000
			2003, 2012		2.792	0.009
			2004, 2005		2.422	0.019
			2004, 2009		2.030	0.049
			2004, 2010		5.060	0.000
			2005, 2007		4.615	0.000
			2005, 2010		2.305	0.025
			2007, 2009		2.572	0.015
			2007, 2010		9.980	0.000
			2007, 2011		2.316	0.033
			2007, 2012		2.897	0.009
			2008, 2010		2.902	0.012
			2010, 2012		2.347	0.029
Season x Year	7.173	0.000	2003	Summer, Winter	3.467	0.005
			2004	Summer, Spring	8.307	0.000
				Winter, Spring	2.822	0.012
			2005	Summer, Autumn	5.052	0.000
				Summer, Spring	8.775	0.000
				Autumn, Winter	2.818	0.014
				Autumn, Spring	5.812	0.000
				Winter, Spring	5.158	0.000
			2006	Autumn, Spring	2.314	0.039
			2007	Summer, Autumn	9.345	0.000
				Summer, Spring	8.234	0.002
				Autumn, Winter	7.195	0.000
				Winter, Spring	6.512	0.002
			2009	Summer, Autumn	3.930	0.002
				Summer, Spring	5.473	0.003
			2010	Summer, Winter	6.549	0.009
				Autumn, Winter	5.305	0.016
				Winter, Spring	3.018	0.019
			2011	Summer, Autumn	5.001	0.001
				Summer, Spring	4.177	0.007
			2012	Summer, Autumn	3.604	0.009
				Summer, Winter	7.074	0.002
				Summer, Spring	4.178	0.003
			Summer	2003, 2004	7.955	0.000
				2003, 2007	4.807	0.001

Table 6 (continued) – Results of PERMANOVA main and pairwise tests for NAO index, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2003, 2009	17.098	0.000
				2003, 2010	3.546	0.003
				2003, 2011	16.823	0.000
				2003, 2012	10.181	0.000
				2004, 2005	4.744	0.000
				2004, 2009	23.833	0.001
				2004, 2010	4.833	0.007
				2004, 2011	10.124	0.000
				2004, 2012	4.549	0.003
				2005, 2007	2.992	0.005
				2005, 2009	9.033	0.000
				2005, 2010	2.372	0.021
				2005, 2011	10.459	0.000
				2005, 2012	9.163	0.000
				2007, 2011	7.781	0.002
				2007, 2012	3.281	0.016
				2008, 2011	2.999	0.018
				2008, 2012	2.997	0.017
				2009, 2011	3.105	0.025
				2010, 2011	8.897	0.003
				2010, 2012	3.712	0.009
			Autumn	2003, 2005	2.354	0.031
				2003, 2006	3.373	0.004
				2003, 2007	3.126	0.007
				2005, 2006	2.495	0.022
				2005, 2007	17.131	0.000
				2005, 2009	2.457	0.025
				2005, 2012	2.966	0.013
				2006, 2007	6.835	0.000
				2006, 2009	3.345	0.005
				2006, 2011	2.617	0.023
				2007, 2010	7.978	0.000
				2007, 2011	2.995	0.010
				2007, 2012	8.485	0.000
			Winter	2003, 2010	11.141	0.003
				2004, 2010	3.674	0.004
				2004, 2011	2.358	0.039
				2005, 2010	4.110	0.004
				2006, 2010	2.777	0.030
				2007, 2010	7.271	0.009
				2008, 2010	11.296	0.003
				2009, 2010	5.878	0.013

Table 6 (continued) – Results of PERMANOVA main and pairwise tests for NAO index, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2010, 2012	12.999	0.001
			Spring	2004, 2005	9.795	0.000
				2004, 2008	4.357	0.001
				2004, 2010	8.001	0.000
				2005, 2006	3.981	0.002
				2005, 2007	8.886	0.000
				2005, 2008	3.330	0.009
				2005, 2009	7.025	0.000
				2005, 2010	2.990	0.012
				2005, 2011	4.815	0.002
				2005, 2012	4.549	0.001
				2007, 2008	6.914	0.002
				2007, 2010	8.736	0.000
				2009, 2010	4.993	0.001
				2010, 2011	3.094	0.017
				2010, 2012	2.341	0.041

Table 7 – Results of PERMANOVA main and pairwise tests for species richness, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Site	13.187	0.000	M, S1		6.149	0.000
			M, S2		3.882	0.004
			M, N1		3.930	0.002
			M, N2		5.653	0.000
			S1, N1		2.847	0.013
			N1, N2		2.994	0.015
Season x Year	1.586	0.035	2003	Summer, Winter	2.625	0.011
			2004	Summer, Winter	2.274	0.029
				Summer, Spring	2.374	0.025
			2007	Autumn, Spring	2.263	0.040
			2008	Summer, Spring	4.679	0.009
			2009	Autumn, Winter	2.219	0.041
				Winter, Spring	9.966	0.004
			2010	Autumn, Spring	3.515	0.016
				Winter, Spring	3.253	0.009
			2011	Summer, Winter	4.026	0.008
				Autumn, Winter	3.040	0.032
			2012	Summer, Autumn	3.040	0.018
				Summer, Winter	2.641	0.043
			Summer	2003, 2004	6.320	0.000
				2003, 2005	2.978	0.005
				2003, 2006	3.114	0.008
				2003, 2007	5.083	0.000
				2003, 2008	4.081	0.001
				2003, 2009	2.700	0.015
				2003, 2010	3.945	0.001
				2003, 2012	2.783	0.011
				2003, 2013	2.452	0.023
				2004, 2008	3.306	0.011
				2004, 2011	4.351	0.002
				2004, 2012	2.678	0.022
				2004, 2013	3.287	0.010
				2007, 2008	3.476	0.018
				2007, 2011	3.358	0.014
				2008, 2011	2.365	0.033
				2010, 2011	2.784	0.028
			Autumn	2003, 2010	3.521	0.004
				2007, 2010	2.438	0.039
				2009, 2010	2.966	0.012
				2010, 2012	4.752	0.024
				2010, 2013	5.149	0.015
				2011, 2012	2.808	0.022

Table 7 (continued) – Results of PERMANOVA main and pairwise tests for species richness, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
			Winter	2004, 2009	2.332	0.027
			Spring	2004, 2007	3.049	0.005
				2004, 2008	2.648	0.010
				2007, 2009	4.223	0.004
				2007, 2010	3.315	0.010
				2007, 2012	3.205	0.016
				2008, 2009	11.161	0.003
				2008, 2010	3.388	0.020
				2010, 2013	2.269	0.046

Table 8 – Results of PERMANOVA main and pairwise tests for Shannon-Wiener index, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Site	5.506	0.0003	M, S1		5.130	0.001
			M, S2		2.645	0.019
			M, N1		2.116	0.042
			M, N2		2.966	0.011
			N1, N2		2.299	0.033
Year	2.309	0.005	2003, 2011		2.628	0.008
			2004, 2013		2.055	0.033
			2005, 2006		3.373	0.001
			2005, 2011		2.425	0.012
			2005, 2012		1.869	0.046
			2006, 2009		2.597	0.008
			2006, 2013		2.911	0.004
			2008, 2013		2.082	0.036
Site x Season	2.022	0.0155	Autumn	N1, N2	2.472	0.021
			Winter	M, S1	9.700	0.000
				M, S2	6.010	0.001
				M, N1	4.098	0.005
				M, N2	3.762	0.007
				S1, S2	2.463	0.035
				S1, N1	5.241	0.001
			Spring	M, S2	3.010	0.010
			M	Summer, Winter	3.119	0.013
				Winter, Spring	2.797	0.026
			S1	Summer, Winter	2.795	0.017
				Winter, Spring	3.490	0.007
			N2	Summer, Autumn	1.843	0.062

Table 9 – Results of PERMANOVA main and pairwise tests for Simpson index, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Year	2.176	0.011	2003, 2011		2.828	0.003
			2004, 2005		2.008	0.034
			2004, 2013		1.993	0.040
			2005, 2006		3.594	0.000
			2005, 2011		3.194	0.001
			2005, 2012		2.477	0.008
			2006, 2009		2.352	0.014
			2006, 2013		2.603	0.007
Site x Year	1.566	0.018	2003	M, S2	3.013	0.009
				S1, S2	4.974	0.002
				S2, N2	4.367	0.002
			2004	S1, N2	4.284	0.002
				N1, N2	2.284	0.033
			2005	M, S1	3.437	0.004
				M, S2	2.645	0.015
				M, N2	2.578	0.014
			2006	M, N1	2.491	0.017
				S1, N1	3.083	0.006
			2009	M, N1	2.563	0.038
			2010	S1, N1	25.214	0.001
				S1, N2	4.579	0.038
				N1, N2	5.613	0.025
			2011	M, N2	2.231	0.040
				S2, N2	5.241	0.003
			2012	S1, N1	2.945	0.027
			2013	S1, N1	2.585	0.038
			M	2003, 2011	2.144	0.039
				2004, 2005	2.501	0.025
				2004, 2012	2.378	0.050
				2005, 2006	2.347	0.032
				2005, 2010	3.334	0.007
				2005, 2011	3.490	0.002
				2005, 2012	4.509	0.001
				2005, 2013	2.456	0.030
				2009, 2010	2.763	0.033
				2009, 2011	2.415	0.023
				2009, 2012	4.855	0.001
				2009, 2013	3.316	0.017
				2012, 2013	3.657	0.011
			S1	2003, 2004	3.207	0.013
				2003, 2012	3.199	0.033
				2004, 2007	2.372	0.044

Table 9 (continued) – Results of PERMANOVA main and pairwise tests for Simpson index, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2004, 2008	4.302	0.007
				2004, 2010	4.043	0.014
				2004, 2013	3.399	0.012
				2005, 2012	2.212	0.042
				2008, 2010	6.181	0.014
			S2	2003, 2005	2.303	0.027
				2003, 2007	2.560	0.023
				2003, 2009	2.408	0.024
				2003, 2013	2.132	0.050
				2007, 2011	3.825	0.007
				2008, 2011	4.770	0.008
				2009, 2011	2.626	0.030
				2011, 2013	2.611	0.038
			N1	2003, 2006	3.005	0.006
				2004, 2005	2.228	0.033
				2005, 2006	3.967	0.001
				2006, 2009	3.454	0.003
				2006, 2010	2.664	0.023
				2006, 2012	3.517	0.004
				2006, 2013	3.957	0.003
			N2	2003, 2008	2.639	0.013
				2004, 2008	2.309	0.043
				2004, 2011	2.324	0.050
				2010, 2011	3.218	0.018

Table 10 – Results of PERMANOVA main and pairwise tests for Pielou's evenness index, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Site	4.792	0.001	M, S1		3.457	0.005
			S1, S2		4.597	0.001
			S1, N1		2.746	0.016
			S1, N2		2.609	0.023
Season	4.293	0.005	Summer, Autumn		2.173	0.041
			Summer, Winter		2.771	0.008
			Summer, Spring		2.618	0.005
Year	2.311	0.010	2003, 2005		2.523	0.011
			2003, 2011		2.288	0.020
			2004, 2005		2.162	0.026
			2005, 2006		3.041	0.002
			2005, 2009		2.030	0.050
			2005, 2011		4.084	0.000
			2005, 2012		2.494	0.013
			2007, 2011		2.218	0.024
			2010, 2011		2.759	0.004
			2011, 2013		1.949	0.045
Site x Year	1.869	0.002	2003	M, S1	3.448	0.006
				S1, S2	6.513	0.001
				S1, N2	2.400	0.049
				S2, N2	4.577	0.002
			2004	N1, N2	2.247	0.037
			2005	M, S2	2.707	0.014
				S1, S2	4.560	0.001
				S1, N2	2.134	0.043
				S2, N1	2.452	0.023
			2006	S1, N1	5.094	0.001
				N1, N2	2.489	0.019
			2007	S1, S2	3.481	0.014
			2008	S1, S2	5.592	0.028
			2009	S1, N1	3.074	0.019
			2010	M, N2	3.035	0.045
			2011	M, N2	2.589	0.022
				S2, N2	9.070	0.000
			2013	M, S1	3.294	0.021
			M	2003, 2005	2.715	0.012
				2005, 2006	2.441	0.030
				2005, 2010	2.905	0.014
				2005, 2011	3.113	0.004
			S1	2003, 2012	2.791	0.034
				2004, 2005	2.756	0.020
				2004, 2007	2.855	0.031

Table 10 (continued) – Results of PERMANOVA main and pairwise tests for Pielou's evenness index, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2004, 2008	2.905	0.034
				2005, 2011	3.132	0.013
				2005, 2012	4.765	0.002
				2006, 2008	3.682	0.016
				2006, 2009	2.726	0.038
				2006, 2012	3.923	0.014
				2007, 2009	3.139	0.025
				2007, 2012	3.456	0.021
				2008, 2009	4.803	0.010
				2009, 2010	3.840	0.025
				2012, 2013	3.386	0.015
			S2	2003, 2005	2.514	0.014
				2003, 2007	2.465	0.029
				2003, 2009	2.430	0.027
				2003, 2010	2.270	0.040
				2003, 2011	2.444	0.049
				2007, 2011	3.451	0.010
				2008, 2011	4.192	0.012
				2009, 2011	2.401	0.048
				2011, 2013	2.722	0.030
			N1	2004, 2005	2.292	0.030
				2005, 2006	3.807	0.001
				2005, 2011	2.711	0.023
				2006, 2007	2.171	0.034
				2006, 2009	3.158	0.004
				2006, 2010	2.678	0.016
				2006, 2012	2.951	0.009
				2006, 2013	3.684	0.003
				2008, 2013	2.706	0.042
			N2	2003, 2011	2.675	0.043
				2005, 2009	2.346	0.045

Table 11 – Results of PERMANOVA main and pairwise tests for functionally singular species (FSing), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Site	13.187	0.000	M, S1		6.149	0.000
			M, S2		3.882	0.004
			M, N1		3.930	0.002
			M, N2		5.653	0.000
			S1, N1		2.847	0.013
			N1, N2		2.994	0.015
Season x Year	1.586	0.035	2003	Summer, Winter	2.625	0.011
			2004	Summer, Winter	2.274	0.029
				Summer, Spring	2.374	0.025
			2007	Autumn, Spring	2.263	0.040
			2008	Summer, Spring	4.679	0.009
			2009	Autumn, Winter	2.219	0.041
				Winter, Spring	9.966	0.004
			2010	Autumn, Spring	3.515	0.016
				Winter, Spring	3.253	0.009
			2011	Summer, Winter	4.026	0.008
				Autumn, Winter	3.040	0.032
			2012	Summer, Autumn	3.040	0.018
				Summer, Winter	2.641	0.043
			Summer	2003, 2004	6.320	0.000
				2003, 2005	2.978	0.005
				2003, 2006	3.114	0.008
				2003, 2007	5.083	0.000
				2003, 2008	4.081	0.001
				2003, 2009	2.700	0.015
				2003, 2010	3.945	0.001
				2003, 2012	2.783	0.011
				2003, 2013	2.452	0.023
				2004, 2008	3.306	0.011
				2004, 2011	4.351	0.002
				2004, 2012	2.678	0.022
				2004, 2013	3.287	0.010
				2007, 2008	3.476	0.018
				2007, 2011	3.358	0.014
				2008, 2011	2.365	0.033
				2010, 2011	2.784	0.028
			Autumn	2003, 2010	3.521	0.004
				2007, 2010	2.438	0.039
				2009, 2010	2.966	0.012
				2010, 2012	4.752	0.024
				2010, 2013	5.149	0.015
				2011, 2012	2.808	0.022

Table 11 (continued) – Results of PERMANOVA main and pairwise tests for functionally singular species (FSing), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
			Winter	2004, 2009	2.332	0.027
			Spring	2004, 2007	3.049	0.005
				2004, 2008	2.648	0.010
				2007, 2009	4.223	0.004
				2007, 2010	3.315	0.010
				2007, 2012	3.205	0.016
				2008, 2009	11.161	0.003
				2008, 2010	3.388	0.020
				2010, 2013	2.269	0.046

Table 12 – Results of PERMANOVA main and pairwise tests for functional richness (FRic), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Site	9.096	0.000	M, S1		3.265	0.002
			M, S2		3.079	0.006
			M, N1		3.603	0.003
			M, N2		5.874	0.000
			S2, N2		3.296	0.003
			N1, N2		2.964	0.002
Year	1.700	0.019	2003, 2005		1.531	0.071
			2005, 2010		1.970	0.010
			2005, 2011		1.770	0.027
			2005, 2013		2.114	0.005
			2008, 2012		2.231	0.022
			2009, 2011		1.722	0.049
			2012, 2013		2.490	0.004
Site x Year	1.405	0.010	M	2003, 2004	2.089	0.024
				2003, 2010	2.733	0.024
				2003, 2011	2.456	0.010
				2004, 2008	2.286	0.013
				2005, 2011	2.059	0.029
				2008, 2012	3.174	0.025
			S1	2004, 2006	2.584	0.046
				2004, 2007	2.132	0.042
				2004, 2008	3.460	0.016
				2004, 2013	8.021	0.001
				2005, 2013	2.187	0.043
				2006, 2013	5.345	0.001
				2010, 2013	5.243	0.021
				2011, 2013	3.699	0.004
				2012, 2013	2.264	0.035
			S2	2011, 2013	2.835	0.010
			N1	2007, 2011	2.572	0.019
				2007, 2012	3.468	0.008
				2008, 2012	7.282	0.007
				2009, 2012	2.538	0.032
				2010, 2012	3.911	0.017
			N2	2003, 2008	5.284	0.004
				2003, 2010	2.718	0.036
				2004, 2005	1.737	0.047
				2005, 2013	2.136	0.027
				2008, 2010	3.240	0.032
				2008, 2012	3.230	0.012
				2008, 2013	2.734	0.016
				2010, 2012	2.431	0.031

Table 12 (continued) – Results of PERMANOVA main and pairwise tests for functional richness (FRic), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2012, 2013	2.252	0.039
			2003	M, S1	2.926	0.012
				M, S2	2.979	0.003
				M, N1	2.151	0.031
				M, N2	5.944	0.000
				N1, N2	2.155	0.021
			2004	M, N1	2.143	0.035
				M, N2	1.742	0.036
			2005	M, N1	1.749	0.048
				M, N2	3.686	0.000
				S1, N2	2.275	0.011
				S2, N2	2.895	0.001
				N1, N2	2.079	0.019
			2007	M, S1	2.451	0.040
				M, S2	3.450	0.004
				M, N1	3.949	0.001
				M, N2	3.448	0.011
				S2, N1	2.153	0.043
			2008	M, S1	3.559	0.046
				M, N1	5.615	0.019
				M, N2	9.809	0.002
				S1, S2	1.661	0.021
				S1, N2	5.283	0.020
			2009	M, N1	2.745	0.007
				M, N2	3.220	0.003
			2010	M, N1	2.566	0.032
				M, N2	3.203	0.014
			2011	S1, N2	2.535	0.010
				S2, N2	4.140	0.003
				N1, N2	2.409	0.022
			2012	M, N2	5.983	0.000
				S1, N2	1.994	0.047
				S2, N2	5.208	0.001
				N1, N2	14.486	0.000
			2013	M, S1	3.357	0.008
				M, N2	2.839	0.009
				S1, S2	4.763	0.002
				S1, N1	3.150	0.015
				S2, N2	2.035	0.019
				N1, N2	2.619	0.012
Season x Year	1.555	0.005	Summer	2003, 2007	3.317	0.001
				2003, 2008	2.465	0.003

Table 12 (continued) – Results of PERMANOVA main and pairwise tests for functional richness (FRic), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2003, 2010	2.778	0.015
				2007, 2012	3.412	0.009
				2007, 2013	2.327	0.022
				2008, 2010	2.166	0.038
				2008, 2012	2.544	0.005
				2010, 2012	2.278	0.028
			Autumn	2003, 2005	1.884	0.017
				2005, 2006	2.125	0.005
				2005, 2007	1.837	0.008
				2005, 2010	1.989	0.013
				2005, 2013	1.995	0.010
				2010, 2013	2.828	0.033
			Winter	2004, 2009	2.153	0.035
			Spring	2005, 2007	1.588	0.097
				2005, 2011	1.975	0.029
				2005, 2013	1.982	0.024
				2007, 2009	2.131	0.018
				2007, 2010	2.269	0.020
				2007, 2011	1.963	0.036
				2007, 2012	2.608	0.030
				2008, 2010	2.328	0.025
				2009, 2013	2.461	0.005
				2010, 2013	2.411	0.010
				2011, 2012	2.286	0.032
				2011, 2013	2.350	0.018
				2012, 2013	2.578	0.010
			2003	Summer, Autumn	2.572	0.005
			2007	Summer, Autumn	1.789	0.047
			2009	Winter, Spring	2.416	0.014
			2010	Summer, Spring	2.234	0.029
				Autumn, Spring	3.090	0.012
			2011	Autumn, Spring	2.065	0.021
			2012	Summer, Autumn	1.883	0.039
				Summer, Winter	2.659	0.016
			2013	Summer, Spring	1.886	0.026
Site x Season x Year	1.320	0.007				

Table 13 – Results of PERMANOVA main and pairwise tests for functional evenness (FEve), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise		
Factor	pseudo-F	p-perm	Level	t	p-perm
Site	2.799	0.033	M, S1	2.540	0.021
			S1, S2	2.839	0.010
			S1, N1	3.116	0.004
Year	1.985	0.030	2003, 2005	3.068	0.002
			2003, 2009	2.078	0.037
			2004, 2005	1.948	0.042
			2005, 2011	3.300	0.003
			2005, 2012	2.098	0.036
			2009, 2011	2.350	0.024
			2010, 2011	2.768	0.009

Table 14 – Results of PERMANOVA main and pairwise tests for functional divergence (FDiv), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Site	5.553	0.001	M, S2		2.919	0.014
			M, N1		3.809	0.003
			M, N2		2.830	0.015
Site x Season	1.820	0.049	Summer	M, S2	3.021	0.014
				M, N1	3.330	0.009
				M, N2	2.368	0.042
			Autumn	M, S2	3.691	0.007
				M, N1	2.619	0.032
				M, N2	3.414	0.012
				S1, S2	2.830	0.023
				S1, N2	5.006	0.002
				S2, N1	2.578	0.029
				N1, N2	2.448	0.042
			Spring	M, S1	3.433	0.015
				S1, S2	2.447	0.022
			S1	Summer, Winter	3.841	0.034
				Autumn, Spring	5.815	0.002
			S2	Autumn, Winter	2.495	0.030

Table 15 – Results of PERMANOVA main and pairwise tests for functional dispersion (FDis), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Site	9.469	0.000	M, S2		3.452	0.005
			M, N1		4.106	0.000
			M, N2		4.060	0.002
			S1, S2		2.609	0.008
			S1, N1		2.037	0.041
			S1, N2		3.741	0.001
			S2, N2		2.315	0.030
			N1, N2		2.796	0.009
Year	1.646	0.044	2003, 2011		2.190	0.024
			2004, 2010		1.803	0.048
			2005, 2013		2.010	0.021
			2007, 2013		2.057	0.025
			2009, 2010		2.727	0.004
			2009, 2012		1.821	0.044
			2010, 2011		2.245	0.018
			2010, 2013		2.310	0.021
Site x Season	1.970	0.008	Summer	M, N2	2.134	0.045
				S1, S2	2.368	0.031
				S1, N1	2.727	0.011
				S1, N2	4.410	0.001
				S2, N1	0.737	0.520
				S2, N2	2.498	0.014
			Autumn	M, S2	2.637	0.032
				M, N2	3.212	0.016
				S1, N2	3.477	0.006
				N1, N2	2.476	0.031
			Winter	M, S2	2.562	0.021
				M, N1	3.950	0.003
				M, N2	2.193	0.048
			Spring	M, S2	3.939	0.001
				M, N1	2.923	0.018
				M, N2	3.783	0.002
				S1, S2	4.043	0.002
				S1, N1	4.168	0.002
				S1, N2	4.082	0.003
				S2, N2	2.842	0.015
				N1, N2	3.061	0.010
			M	Summer, Autumn	1.991	0.045
			S1	Autumn, Spring	2.693	0.018
				Winter, Spring	3.021	0.032
			N1	Autumn, Winter	2.525	0.033
Site x Year	2.071	0.000	M	2003, 2011	3.641	0.007

Table 15 (continued) – Results of PERMANOVA main and pairwise tests for functional dispersion (FDis), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2004, 2005	3.338	0.003
				2004, 2012	3.624	0.007
				2005, 2011	4.523	0.000
				2005, 2013	2.097	0.050
				2007, 2011	2.639	0.019
				2008, 2011	2.656	0.021
				2009, 2011	3.304	0.008
				2009, 2012	3.096	0.027
				2011, 2012	2.698	0.021
				2011, 2013	2.635	0.020
			S1	2003, 2006	2.494	0.049
				2003, 2012	3.129	0.017
				2003, 2013	2.688	0.031
				2006, 2007	3.864	0.041
				2006, 2008	6.226	0.034
				2006, 2011	3.674	0.022
				2006, 2012	3.017	0.043
				2007, 2008	4.631	0.038
				2011, 2012	2.322	0.044
			N1	2004, 2005	2.015	0.039
				2004, 2012	2.016	0.047
				2004, 2013	2.212	0.031
				2007, 2010	2.125	0.044
				2008, 2010	11.973	0.001
			N2	2003, 2010	2.506	0.042
				2004, 2009	2.373	0.021
				2004, 2010	3.440	0.010
				2004, 2011	2.131	0.043
				2005, 2009	2.387	0.011
				2005, 2010	3.564	0.001
				2005, 2011	2.117	0.046
				2005, 2012	3.090	0.008
				2007, 2013	2.742	0.047
				2009, 2010	3.761	0.004
				2009, 2011	2.185	0.033
				2009, 2012	3.763	0.008
				2010, 2011	3.631	0.016
				2010, 2013	4.100	0.002
				2011, 2013	2.464	0.030
				2012, 2013	3.512	0.012
			2003	M, S2	2.912	0.008
				M, N1	2.176	0.040

Table 15 (continued) – Results of PERMANOVA main and pairwise tests for functional dispersion (FDis), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				M, N2	4.488	0.003
				S1, S2	5.140	0.001
				S1, N2	5.402	0.004
				N1, N2	4.298	0.002
			2004	M, S1	2.437	0.033
				M, N1	2.182	0.027
				M, N2	2.492	0.015
				S1, N1	2.327	0.025
				S1, N2	3.416	0.004
			2005	M, S2	3.280	0.002
				M, N1	2.688	0.014
				M, N2	8.033	0.000
				S1, N2	4.583	0.000
				S2, N2	4.800	0.000
				N1, N2	4.782	0.000
			2006	S1, N2	17.561	0.039
			2007	M, N1	3.117	0.014
				S1, N1	3.655	0.011
			2008	S1, N1	65.788	0.033
			2009	M, S1	2.600	0.010
				M, S2	3.406	0.010
				M, N1	3.893	0.007
				M, N2	6.595	0.000
				S1, N1	2.746	0.022
				S1, N2	3.916	0.009
				S2, N2	3.158	0.004
				N1, N2	4.280	0.001
			2010	S1, N1	28.623	0.002
			2011	M, S1	2.574	0.029
				S1, S2	2.873	0.028
				S1, N2	4.364	0.005
				S2, N2	3.245	0.016
			2012	M, S2	3.756	0.014
				M, N2	3.662	0.008
				N1, N2	2.622	0.047
			2013	M, S2	2.832	0.023
				M, N2	5.222	0.001
				S1, N2	4.273	0.003
				S2, N2	4.602	0.003
				N1, N2	4.872	0.002
Season x Year	1.443	0.037	2004	Winter, Spring	1.854	0.040
			2005	Autumn, Winter	1.911	0.029

Table 15 (continued) – Results of PERMANOVA main and pairwise tests for functional dispersion (FDis), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
			2009	Autumn, Winter	2.941	0.004
				Autumn, Spring	1.853	0.032
				Winter, Spring	2.432	0.021
			2012	Summer, Winter	4.422	0.010
				Winter, Spring	3.606	0.016
			Summer	2003, 2005	2.144	0.026
				2003, 2010	2.389	0.021
				2004, 2012	2.114	0.039
				2005, 2006	2.012	0.028
				2010, 2013	3.496	0.011
			Autumn	2003, 2011	1.902	0.038
				2003, 2012	1.774	0.047
				2003, 2013	1.911	0.034
				2005, 2011	2.177	0.013
				2005, 2013	1.947	0.032
				2007, 2011	2.201	0.024
				2007, 2013	2.194	0.023
			Winter	2004, 2009	2.162	0.030
				2005, 2008	2.336	0.032
				2005, 2009	3.291	0.008
			Spring	2004, 2010	2.048	0.021
				2004, 2012	2.114	0.019
				2005, 2006	2.175	0.033
				2005, 2011	2.309	0.020
				2006, 2007	2.019	0.047
				2006, 2009	2.078	0.040
				2006, 2012	2.979	0.010
				2009, 2011	2.991	0.005
				2009, 2012	2.132	0.011
				2009, 2013	2.019	0.026
				2010, 2011	2.667	0.024
				2011, 2012	3.655	0.004
				2012, 2013	2.475	0.018
Site x Season x Year	1.358	0.015				

Table 16 – Results of PERMANOVA main and pairwise tests for functional Rao's Quadratic Entropy (FRaoQ), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Site	11.004	0.000	M, S2		3.700	0.002
			M, N1		4.142	0.001
			M, N2		4.755	0.000
			S1, S2		2.286	0.014
			S1, N1		2.418	0.015
			S1, N2		3.432	0.000
			S2, N2		2.720	0.009
			N1, N2		2.733	0.006
Site x Season	1.692	0.023	Summer	M, N2	3.187	0.005
				S1, S2	2.194	0.030
				S1, N1	3.014	0.010
				S1, N2	6.014	0.000
				S2, N2	3.797	0.001
				N1, N2	2.328	0.022
			Autumn	M, N2	3.562	0.006
				S1, N2	3.743	0.003
				S2, N2	3.018	0.007
				N1, N2	2.566	0.021
			Winter	M, S2	2.702	0.009
				M, N1	4.049	0.001
			Spring	M, S2	3.971	0.001
				M, N1	3.319	0.009
				M, N2	3.477	0.001
				S1, S2	3.923	0.001
				S1, N1	2.947	0.011
				S1, N2	3.680	0.002
				S2, N2	2.469	0.017
				N1, N2	2.249	0.022
			S1	Autumn, Spring	2.671	0.009
				Winter, Spring	2.613	0.036
			N1	Autumn, Winter	2.497	0.020
Site x Year	1.583	0.002	2003	M, S2	2.565	0.016
				M, N2	4.303	0.002
				S1, S2	3.328	0.011
				S1, N2	4.311	0.005
				N1, N2	3.687	0.002
			2004	M, S1	2.384	0.032
				S1, N2	2.184	0.015
			2005	M, S2	2.878	0.003
				M, N1	2.025	0.038
				M, N2	6.325	0.000
				S1, N2	3.383	0.001

Table 16 (continued) – Results of PERMANOVA main and pairwise tests for functional Rao's Quadratic Entropy (FRaoQ), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				S2, N2	4.198	0.000
				N1, N2	3.638	0.000
			2006	S1, N2	28.214	0.039
			2007	M, S2	2.278	0.042
				M, N1	5.256	0.000
				M, N2	2.452	0.033
				S1, N1	4.405	0.005
				S2, N1	2.312	0.033
			2008	M, N1	4.788	0.017
				M, N2	2.389	0.047
				S1, N1	12.271	0.034
			2009	M, S1	4.448	0.004
				M, S2	2.474	0.047
				M, N1	4.120	0.004
				M, N2	3.995	0.001
				S1, N1	3.231	0.012
				S1, N2	2.436	0.033
				S2, N2	1.995	0.034
				N1, N2	2.275	0.013
			2010	S1, N1	5.502	0.007
			2011	M, S1	2.614	0.030
				S1, N2	5.225	0.001
				S2, N2	3.558	0.009
			2012	M, S2	6.079	0.001
				M, N2	6.023	0.001
				S2, N2	5.004	0.003
				N1, N2	4.091	0.010
			2013	M, S2	3.610	0.005
				M, N1	2.608	0.013
				M, N2	9.119	0.000
				S1, N2	3.515	0.021
				S2, N2	6.524	0.001
				N1, N2	6.098	0.001
			M	2003, 2011	4.022	0.002
				2003, 2012	2.800	0.045
				2004, 2005	2.800	0.008
				2004, 2007	2.490	0.018
				2004, 2008	2.372	0.033
				2004, 2012	3.798	0.004
				2005, 2006	3.032	0.004
				2005, 2009	2.415	0.027
				2005, 2011	3.706	0.001

Table 16 (continued) – Results of PERMANOVA main and pairwise tests for functional Rao's Quadratic Entropy (FRaoQ), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2006, 2007	2.505	0.017
				2007, 2009	2.457	0.037
				2007, 2011	2.978	0.007
				2008, 2009	2.659	0.027
				2008, 2011	2.593	0.019
				2009, 2011	2.828	0.018
				2009, 2012	4.475	0.004
				2011, 2012	2.502	0.016
				2011, 2013	2.462	0.028
			S1	2003, 2007	2.185	0.033
				2006, 2008	9.231	0.035
				2006, 2011	3.952	0.014
				2007, 2008	3.699	0.038
				2009, 2010	2.346	0.040
				2009, 2011	3.646	0.015
				2010, 2011	2.352	0.034
			N1	2003, 2009	2.027	0.048
				2007, 2010	5.490	0.002
				2007, 2012	3.156	0.016
				2007, 2013	2.553	0.028
				2008, 2010	4.841	0.049
			N2	2005, 2010	2.683	0.003
				2005, 2011	1.816	0.046
				2005, 2012	2.014	0.045
				2006, 2013	3.465	0.030
				2007, 2013	5.763	0.014
				2009, 2010	2.052	0.046
				2009, 2012	2.187	0.036
				2010, 2011	2.728	0.033
				2010, 2012	3.114	0.023
				2010, 2013	5.923	0.003
				2012, 2013	3.237	0.024

Table 17 – Results of PERMANOVA main and pairwise tests for phylogenetic Rao's Quadratic Entropy (PRaoQ), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Year	2.377	0.005	2003, 2011		2.833	0.004
			2003, 2012		1.974	0.049
			2004, 2005		2.445	0.008
			2005, 2006		4.036	0.000
			2005, 2007		2.175	0.027
			2005, 2008		1.997	0.038
			2005, 2011		4.082	0.000
			2005, 2012		3.408	0.001
			2006, 2009		2.118	0.033
Site x Season	2.295	0.006	Winter	M, S1	6.479	0.000
				M, S2	4.284	0.003
				M, N1	2.681	0.023
				M, N2	2.192	0.028
				S1, S2	2.849	0.015
				S1, N1	3.271	0.010
			M	Summer, Winter	3.781	0.005
				Winter, Spring	3.254	0.012
			S1	Summer, Winter	2.672	0.019
				Autumn, Winter	2.501	0.029
				Winter, Spring	3.689	0.005
			N1	Summer, Autumn	1.668	0.050
Site x Year	1.522	0.022	2003	M, S2	2.190	0.035
				S1, S2	4.590	0.001
				S2, N2	4.754	0.002
			2004	M, N2	2.427	0.033
				S1, N2	3.852	0.005
				N1, N2	2.360	0.028
			2005	M, S1	3.867	0.002
				M, S2	2.744	0.016
				M, N1	2.207	0.038
				M, N2	2.724	0.009
			2006	S1, N1	3.163	0.008
			2010	S1, N1	10.596	0.001
				S1, N2	7.182	0.006
			2011	M, N2	2.257	0.035
				S2, N2	4.125	0.005
			2012	S1, N1	3.072	0.023
			M	2003, 2005	2.724	0.014
				2004, 2005	3.809	0.002
				2004, 2012	2.827	0.025
				2005, 2006	3.103	0.005
				2005, 2010	4.004	0.004

Table 17 (continued) – Results of PERMANOVA main and pairwise tests for phylogenetic Rao's Quadratic Entropy (PRaoQ), considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2005, 2011	3.582	0.004
				2005, 2012	4.287	0.002
				2005, 2013	4.373	0.001
				2009, 2011	2.122	0.041
			S1	2003, 2012	3.456	0.021
				2005, 2012	3.748	0.004
				2005, 2013	2.113	0.043
				2006, 2012	3.167	0.024
			S2	2003, 2005	1.996	0.049
				2003, 2007	2.632	0.018
				2003, 2009	2.476	0.018
				2007, 2011	3.432	0.012
				2007, 2012	2.592	0.024
				2008, 2011	3.764	0.020
				2009, 2011	2.404	0.043
			N1	2003, 2006	3.178	0.005
				2004, 2005	2.226	0.030
				2005, 2006	4.799	0.001
				2005, 2011	2.647	0.025
				2006, 2007	2.356	0.031
				2006, 2009	4.061	0.002
				2006, 2010	3.123	0.011
				2006, 2012	3.346	0.005
				2006, 2013	4.320	0.002
			N2	2003, 2008	2.672	0.013
				2004, 2008	2.283	0.043

Table 18 – Results of PERMANOVA main and pairwise tests for phylogenetic mean pairwise distance, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
Site	7.295	0.000	M, S1		3.146	0.008
			M, N1		3.217	0.010
			S1, S2		3.160	0.008
			S1, N2		3.112	0.011
			S2, N1		4.061	0.003
			N1, N2		3.054	0.012
Year	3.195	0.007	2003, 2010		3.068	0.005
			2003, 2013		2.704	0.021
			2004, 2010		2.826	0.009
			2004, 2013		3.994	0.000
			2005, 2007		2.290	0.034
			2005, 2010		3.255	0.002
			2005, 2012		2.250	0.030
			2005, 2013		5.483	0.000
			2006, 2010		2.052	0.049
			2006, 2013		4.502	0.000
			2010, 2013		2.308	0.049
			2011, 2013		3.711	0.001
			2012, 2013		2.474	0.024
Site x Season	3.397	0.000	Summer	S1, S2	3.438	0.005
				S2, N1	4.871	0.001
				S2, N2	4.054	0.003
			Autumn	M, S1	2.416	0.041
				M, N1	2.483	0.040
				S1, S2	2.709	0.025
				S1, N2	2.362	0.045
			Winter	M, S1	3.041	0.011
				M, S2	2.275	0.061
				M, N1	2.445	0.027
				S1, S2	2.564	0.027
				S1, N1	2.656	0.022
				S1, N2	2.239	0.035
			Spring	M, S2	3.797	0.005
				M, N2	2.350	0.048
				S2, N1	4.655	0.001
				N1, N2	2.843	0.024
			M	Winter, Spring	2.993	0.020
			S1	Winter, Spring	2.698	0.018
			S2	Summer, Winter	2.684	0.027
				Autumn, Spring	2.736	0.023

Table 17 (continued) – Results of PERMANOVA main and pairwise tests for phylogenetic mean pairwise distance, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test Factor	pseudo-F	p-perm	Pairwise Level		t	p-perm
				Winter, Spring	4.328	0.002
			N2	Summer, Spring	3.520	0.007
				Autumn, Spring	2.684	0.037
Site x Year	1.936	0.021	2003	M, N1	4.089	0.003
				N1, N2	4.626	0.002
			2006	M, S2	2.701	0.024
				S2, N1	3.454	0.009
				N1, N2	2.781	0.030
			2008	S2, N1	5.119	0.038
				N1, N2	7.861	0.010
			2010	S1, N2	9.050	0.003
			2011	S1, S2	3.098	0.024
				S2, N1	2.675	0.039
				S2, N2	4.405	0.007
			2012	M, S1	8.857	0.001
				M, N2	4.012	0.016
				S1, N2	3.161	0.022
			M	2003, 2009	4.055	0.004
				2003, 2013	2.488	0.039
				2005, 2006	2.202	0.046
				2006, 2012	2.366	0.049
				2009, 2012	3.716	0.015
				2012, 2013	4.195	0.021
			S1	2004, 2008	2.620	0.046
				2004, 2010	3.350	0.033
				2004, 2013	2.757	0.025
				2005, 2007	2.644	0.035
				2005, 2008	3.207	0.010
				2005, 2010	4.064	0.003
				2005, 2012	4.658	0.001
				2005, 2013	5.183	0.001
				2006, 2013	3.373	0.017
				2008, 2011	3.933	0.030
				2010, 2011	7.958	0.028
				2011, 2012	9.261	0.001
				2011, 2013	3.235	0.022
			S2	2006, 2013	4.156	0.005
				2007, 2013	3.175	0.017
				2011, 2013	3.751	0.011
			N2	2003, 2008	3.426	0.022
				2003, 2009	2.563	0.042

Table 17 (continued) – Results of PERMANOVA main and pairwise tests for phylogenetic mean pairwise distance, considering the factors site, season and year, including interactions. Only significant results are presented (p-value<0.05).

Main test			Pairwise			
Factor	pseudo-F	p-perm	Level		t	p-perm
				2003, 2010	5.193	0.020
				2003, 2011	3.303	0.023
				2003, 2012	3.362	0.015
				2006, 2012	3.206	0.021
				2007, 2009	3.117	0.020
				2007, 2010	4.402	0.008
				2007, 2011	4.184	0.009
				2007, 2012	3.312	0.022
				2010, 2011	3.025	0.034
				2011, 2012	3.022	0.032